

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.

THIS PAGE BLANK (USPTO)



INTERNATIONAL APPLICATION PUBLISHED UNDER

WO 9607739A2

(51) International Patent Classification 6: C12N 15/12, C07K 14/715, 16/28, A61K 38/17, C12Q 1/68		(A2)	(11) International Publication Number: WO 96/07739 (43) International Publication Date: 14 March 1996 (14.03.96)
(21) International Application Number: PCT/US95/12037		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SL, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
(22) International Filing Date: 11 September 1995 (11.09.95)		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(30) Priority Data: 08/303,957 9 September 1994 (09.09.94) US			
(71) Applicant: NEUROCRINE BIOSCIENCES, INCORPORATED (US/US); 3050 Science Park Road, San Diego, CA 92121 (US).			
(72) Inventors: LOVENBERG, Timothy, W.; 6529 Vispera Place, Carlsbad, CA 92009 (US); OLTENSDORF, Tilman; 427 Bristol Avenue, Cardiff, CA 92007 (US); LIAW, Chen, W.; 7668 Salix Place, San Diego, CA 92129 (US); CLEVENGER, William; 1510 South Melrose Drive #7, Vista, CA 92083 (US); DeSOUZA, Errol, B.; 4507 South Lane, Del Mar, CA 92014 (US).			
(74) Agents: McMASTER, David, D. et al; Seed and Berry, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).			
(54) Title: INTERLEUKIN-1 TYPE 3 RECEPTORS			
(57) Abstract			
<p>The present invention provides isolated nucleic acid molecules encoding soluble and membrane bound forms of Interleukin-1 Type 3 receptors, as well as recombinant expression vectors and host cells suitable for expressing such receptors.</p>			
<p>EXTRACELLULAR DOMAIN aa 1-338</p> <p>TRANSMEMBRANE DOMAIN aa 339 TO 359</p> <p>INTRACELLULAR DOMAIN aa 360 TO 561</p> <p>RAT IL-1 TYPE 3 RECEPTOR</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Beira	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russia Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	L1	Lithuania	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Lithuania	TG	Togo
CZ	Czech Republic	MC	Macao	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Description

INTERLEUKIN-1 TYPE 3 RECEPTORS

5 Technical Field

The present invention relates generally to cell surface receptors, and more specifically, to Interleukin-1 Type 3 receptors.

Background Of The Invention

10 Interleukin-1 ("IL-1") is a cytokine which is known to be a key mediator of immunological and pathological responses to stress, infection and antigenic challenge (Oppenheim et al., *Immunol. Today* 7:45-46, 1986; Dinarello, *FASEB J.* 2:108-115, 1988; and Mizel, *FASEB J.* 3:2379-2388, 1989). In addition, IL-1 is known to have a variety of effects on the brain and central nervous system. For example, IL-1 has been
15 postulated to be involved in the induction of fever (Kluger, *Physiol. Rev.* 71:93-127, 1991), increased duration of slow wave sleep (Opp et al., *Am. J. Physiol.* 260:R52-R58, 1991), decreased appetite (McCarthy et al., *Am. J. Clin. Nutr.* 42:1179-1182, 1985), activation of the hypothalamic-pituitary-adrenal ("HPA") axis (Woloski et al., *Science* 230:1035-1037, 1985), and inhibition of the hypothalamic-pituitary-gonadal axis (River
20 and Vale, *Endocrinology* 124:2105-2109, 1989).

In light of the above-noted effects of IL-1 (as well as many others), substantial effort has been undertaken in order to identify receptors for IL-1. Briefly, at least two types of receptors are known to be expressed on the surface of certain immune cells in both human and murine derived lines. Type I receptors bind both IL-1 α and IL-1 β , and can be found on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes and hepatocytes (U.S. Patent Nos. 4,968,607, 5,081,228, and 5,180,812; Chizzonite et al., *PNAS* 86:8029-8033, 1989; Dinarello et al., *Blood* 77:1627-1652, 1991). Type II receptors can be found on various B cell lines, including the Raji human B-cell lymphoma line (Bomsztyk et al., *PNAS* 86:8034-8038, 1989; Horuk et al., *J. Biol. Chem.* 262:16275-16278, 1987; Horuk and McCubrey, *Biochem. J.* 260:657-663, 1989).

30 The present invention provides new, previously unidentified Interleukin receptors, designated Interleukin-1 Type 3 receptors ("IL-1-3R"). In addition, the present invention provides compositions and methods which utilize such receptors, as well as other, related advantages.

Summary of the Invention

Briefly stated, the present invention provides compositions and methods which comprise Interleukin-1 Type 3 receptors. Within one aspect of the present invention isolated nucleic acid molecules are provided which encode Interleukin-1 Type 5 receptors. Within one embodiment, the isolated nucleic acid molecules comprise the sequence of nucleotides in Sequence I.D. No. 1, from nucleotide number 129 to nucleotide number 1814. Within another embodiment, the isolated nucleic acid molecules encode a protein having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 562. Within other embodiments, 10 isolated nucleic acid molecules are provided in Sequence I.D. No. 3, from nucleotide number 89 to nucleotide number 1771. Within another embodiment, the nucleic acid molecules encode a protein having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 561. Nucleic acid molecules which encode IL-1 Type 3 receptors of the present invention may be isolated from virtually any 15 warm-blooded animal, including for example, humans, macaques, horses, cattle, sheep, pigs, dogs, cats, rats and mice.

Within related aspects of the present invention, isolated nucleic acid molecules are provided which encode soluble Interleukin-1 Type 3 receptors. Within 20 one embodiment, the isolated nucleic acid molecules comprise the sequence of nucleotides in Sequence I.D. No. 1, from nucleotide number 129 to nucleotide number 1136. Within other embodiments, the isolated nucleic acid molecules encode a protein having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 336. Within another embodiment, the nucleic acid molecules comprise the sequence of nucleotides in Sequence I.D. No. 3, from nucleotide 25 number 89 to nucleotide number 1102. Within yet another embodiment, the nucleic acid molecules encode a protein having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 338. As above, nucleic acid molecules which encode soluble IL-1 Type 3 receptors of the present invention may be isolated from virtually any warm-blooded animal, including for example, humans, macaques, 30 horses, cattle, sheep, pigs, dogs, cats, rats and mice.

Within other aspects of the present invention, expression vectors are provided which are capable of expressing the above-described nucleic acid molecules. Within one embodiment, such vectors comprise a promoter operably linked to one of the 35 above-described nucleic acid molecules. Within other embodiments, recombinant viral vectors are provided which are capable of directing the expression of one of the above described nucleic acid molecules. Representative examples of such viral vectors include retroviral vectors, adenoviral vectors, and herpes simplex virus vectors. Also provided

by the present invention are host cells containing one of the above-described recombinant vectors.

Within other aspects of the present invention, isolated Interleukin-1 Type 3 receptors are provided. Within one embodiment, such receptors have the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 562. Within another embodiment, the receptors have the sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 561. Within yet further aspects of the invention, isolated soluble Interleukin-1 Type 3 receptors are provided. Within one embodiment, the isolated soluble Interleukin-1 Type 3 receptors have the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 336. Within another embodiment, the soluble receptors have the sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 338.

Within other aspects of the invention, isolated antibodies capable of specifically binding to an Interleukin-1 Type 3 receptor are provided. Within one embodiment, the antibody may be selected from the group consisting of polyclonal antibodies, monoclonal antibodies, and antibody fragments. Within other embodiments, antibodies are provided which are capable of blocking the binding of IL-1 to an Interleukin-1 Type 3 receptor. Within preferred embodiments, the antibody is selected from the group consisting of murine and human antibodies. In addition to antibodies, the present invention also provides hybridomas which produce an antibody as described above.

Within yet another aspect of the present invention, nucleic acid molecules are provided which are capable of specifically hybridizing to a nucleic acid molecule encoding any of the Interleukin-1 Type 3 receptors described above. Such molecules may be between at least "y" nucleotides long, wherein "y" is any integer between 14 and 2044, and furthermore, may be selected suitable for use as probes or primers described below. Particularly preferred probes of the present invention are at least 18 nucleotides in length.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures or compositions (e.g., plasmids, etc.), and are therefore incorporated by reference in their entirety.

35 **Brief Description of the Drawings**

Figure 1 schematically illustrates a rat IL-1 type 3 receptor.

Figure 2 is a table which lists the homology of a human IL-1 type 3 receptor with its rat homologue, and other interleukin receptors.

Figure 3 is a graph which shows stimulation of a reporter product via a human IL-1 type 3 receptor.

5 Figure 4 is a graph which shows the expression pattern of the IL-1 Type 3 receptor based upon RNA protection assays.

Figures 5A and B are two graphs which show inhibition of thymocyte proliferation by soluble IL-1 receptors.

10 Detailed Description of the Invention

Definitions

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms to be used hereinafter.

15 "Interleukin-1 Type 3 Receptors" ("IL-1-3R") refers to receptor proteins which bind Interleukin-1 (α or β), and, when expressed on a cell surface, transduce the signal provided by Interleukin-1 to the cell, thereby mediating a biological effect within the cell. In their native configuration, IL-1 Type 3 receptors exist as membrane bound proteins, consisting of an extracellular domain, transmembrane domain, and intracellular domain (see Figure 1). IL-1-3R may be distinguished from other Interleukin-1 receptors 20 based upon criteria such as affinity of substrate binding, tissue distribution, and sequence homology. For example, IL-1-3R of the present invention should be greater than 50% homologous, preferably greater than 75% to 80% homologous, more preferably greater than 85% to 90% homologous, and most preferably greater than 92%, 95%, or 97% homologous to the IL-1-3R disclosed herein (e.g., Sequence ID. No. 1). As utilized 25 within the context of the present invention, IL-1-3R should be understood to include not only the proteins which are disclosed herein, but substantially similar derivatives and analogs as discussed below.

30 "Soluble Interleukin-1 Type 3 Receptor" ("sIL1-3R") refers to a protein which has an amino acid sequence corresponding to the extracellular region of an Interleukin-1 Type 3 receptor. The extracellular region of IL-1-3R may be readily determined by a hydrophobicity analysis utilizing a computer program such as PROTEAN (DNASTAR, Madison, WI), or by an alignment analysis with other known type 1 and type 2 Interleukin-1 receptors.

35 "Nucleic acid molecule" refers to a nucleic acid polymer or nucleic acid sequence, which exists in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule must have been derived from nucleic acids isolated at least once in substantially pure form, (i.e., substantially free of

contaminating endogenous materials), and in a quantity or concentration enabling identification and recovery. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns. As utilized herein, nucleic acid molecules should be understood to include deoxyribonucleic acid ("DNA") molecules (including genomic and cDNA molecules), ribonucleic acid ("RNA") molecules, hybrid or chimeric nucleic acid molecules (e.g., DNA-RNA hybrids), and where appropriate, nucleic acid molecule analogs and derivatives (e.g., peptide nucleic acids ("PNA")). Nucleic acid molecules of the present invention may also comprise sequences of non-translated nucleic acids where such additional sequences 5 do not interfere with manipulation or expression of the open reading frame (e.g., sequences which are 5' or 3' from the open reading frame).

10 15 "Recombinant expression vector" refers to a replicable nucleic acid construct used either to amplify or to express nucleic acid sequences which encode IL-1 Type 3, or sIL-1 Type 3 receptors. This construct comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters, and (2) the structural or coding sequence of interest. The recombinant expression vector may also comprise appropriate transcription and translation initiation and termination sequences.

20 As noted above, the present invention provides isolated nucleic acid molecules encoding Interleukin-1 Type 3 receptors. One representative IL-1 Type 3 receptor which may be obtained utilizing the methods described herein (see, e.g., Example 1) is schematically illustrated in Figure 1. Briefly, this IL-1 Type 3 receptor (see Sequence I.D. Nos. 1 and 2) is composed of an Extracellular N-terminal Domain 25 (amino acids 1 - 336), a Transmembrane Domain (amino acids 337 - 357), and a C-terminal Intracellular Domain (358 - 562).

30 35 Although the above IL-1 Type 3 receptor has been provided for purposes of illustration (see also Sequence I.D. Nos. 3 and 4), the present invention should not be so limited. In particular, "IL-1-3R" and "sIL-1-3R" as utilized herein should be understood to include a wide variety of IL-1 Type 3 receptors which are encoded by nucleic acid molecules that have substantial similarity to the sequences disclosed in Sequences I.D. Nos. 1 and 3. As utilized within the context of the present invention, nucleic acid molecules which encode IL-1 Type 3 receptors are deemed to be substantially similar to those disclosed herein if: (a) the nucleic acid sequence is derived from the coding region of a native IL-1 Type 3 receptor gene (including, for example, allelic variations of the sequences disclosed herein); (b) the nucleic acid sequence is capable of hybridization to nucleic acid sequences of the present invention under

conditions of either moderate (e.g., 50% formamide, 5 x SSPE, 5 x Denhardt's, 0.1% SDS, 100 ug/ml Salmon Sperm DNA, and a temperature of 42°C) or high stringency (see Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, NY, 1989); or (c) nucleic acid sequences are degenerate as a result of the genetic code to the nucleic acid sequences defined in (a) or (b). Furthermore, as noted above, although DNA molecules are primarily referred to herein, as should be evident to one of skill in the art given the disclosure provided herein, a wide variety of related nucleic acid molecules may also be utilized in various embodiments described herein, including for example, RNA, nucleic acid analogues, as well as chimeric nucleic acid molecules which may be composed of more than one type of nucleic acid.

In addition, as noted above, within the context of the present invention "IL-1 Type 3 receptors" and "soluble IL-1 Type 3 receptors" should be understood to include derivatives and analogs of the IL-1 Type 3 receptors described above. Such derivatives include allelic variants and genetically engineered variants that contain conservative amino acid substitutions and/or minor additions, substitutions or deletions of amino acids, the net effect of which does not substantially change the biological activity (e.g., signal transduction) or function of the IL-1 Type 3 receptor. Such derivatives are generally greater than about 50% homologous, preferably greater than 75% to 80% homologous, more preferably greater than 85% to 90% homologous, and most preferably greater than 92%, 95% or 97% homologous. Homology may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG).

The primary amino acid structure of IL-1 Type 3 receptors may also be modified by derivatizing amino acid side chains, and/or the amino or carboxy terminus with various functional groups, in order to allow for the formation of various conjugates (e.g., protein-IL-1-3R conjugates). Alternatively, conjugates of IL-1-3R (and sIL-1-3R) may be constructed by recombinantly producing fusion proteins. Such fusion proteins may comprise, for example, IL-1-3R-protein Z wherein protein Z is another cytokine receptor (e.g., IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R, IL-15R or TNF (α or β) receptor; see WO91/03553); a binding portion of an antibody; a toxin (as discussed below); or a protein or peptide which facilitates purification or identification of IL-1-3R (e.g., poly-His). For example, a fusion protein such as human IL-1-3R (His)_n or sIL-1-3R (His)_n may be constructed in order to allow purification of the protein via the poly-His residue, for example, on a NTA nickel-chelating column. The amino acid sequence of a IL-1 Type 3 receptor may

also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (Sequence I.D. No. 5) (Hopp et al., *Bio/Technology* 6:1204, 1988) in order to facilitate purification of expressed recombinant protein.

The present invention also includes IL-1-3R (and sIL-1-3R) proteins which may be produced either with or without associated native-pattern glycosylation. For example, expression of IL-1-3R DNAs in bacteria such as *E. coli* provides non-glycosylated molecules. In contrast, IL-1-3R expressed in yeast or mammalian expression systems (as discussed below) may vary in both glycosylation pattern and molecular weight from native IL-1-3R, depending on the amino acid sequence and expression system which is utilized. In addition, functional mutants of mammalian IL-1-3R having inactivated glycosylation sites may also be produced in a homogeneous, reduced-carbohydrate form, utilizing oligonucleotide synthesis, site-directed mutagenesis, or random mutagenesis techniques. Briefly, N-glycosylation sites in eukaryotic proteins are generally characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. In this triplet, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such sites may be eliminated by deleting Asn or Z, substituting another amino acid for Asn or for residue Z, or inserting a non-Z amino acid between A₁ and Z, or an amino acid other than Asn between Asn and A₁.

Proteins which are substantially similar to IL-1-3R proteins may also be constructed by, for example, substituting or deleting various amino acid residues which are not required for biological activity. For example, cysteine residues may be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Similarly, adjacent dibasic amino acid residues may be modified for expression in yeast systems in which KEX2 protease activity is present.

Not all mutations in the nucleotide sequence which encodes IL-1-3R will be expressed in the final product. For example, nucleotide substitutions may be made in order to avoid secondary structure loops in the transcribed mRNA, or to provide codons that are more readily translated by the selected host, and thereby enhance expression within a selected host.

Generally, substitutions at the amino acid level should be made conservatively, i.e., the most preferred substitute amino acids are those which have characteristics resembling those of the residue to be replaced. When a substitution, deletion, or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered utilizing, for example, the signalling assay disclosed within the Examples.

Mutations which are made to the sequence of the nucleic acid molecules of the present invention should generally preserve the reading frame phase of the coding sequences. Furthermore, the mutations should preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon, and the expressed IL-1-3R mutants screened for the biological activity. Representative methods for random mutagenesis include those described by Ladner et al. in U.S. Patent Nos. 5,096,815; 5,198,346; and 5,223,409.

As noted above, mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, site-directed mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik, *Bio Techniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Sambrook et al. (*Molecular cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, 1989); and U.S. Patent Nos. 4,518,584 and 4,737,462, which are incorporated by reference herein.

IL-1 Type 3 receptors, as well as substantially similar derivatives or analogs may be used as therapeutic reagents, immunogens, reagents in receptor-based immunoassays, or as binding agents for affinity purification procedures. Moreover, IL-1 Type 3 receptors of the present invention may be utilized to screen compounds for IL-1 Type 3 receptor agonist or antagonistic activity. IL-1 Type 3 receptor proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromine-activated, bisoxirane-activated, carbonyldiimidazole-activated, or tosyl-activated, agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, IL-1-3R may be used to selectively bind (for purposes of assay or purification) anti-IL-1-3R antibodies or IL-1.

ISOLATION OF IL-1 TYPE 3 RECEPTOR cDNA CLONES

As noted above, the present invention provides isolated nucleic acid molecules which encode IL-1 Type 3 receptors. Briefly, nucleic acid molecules which encode IL-1 Type 3 receptors of the present invention may be readily isolated from a variety of warm-blooded animals, including for example, humans, macaques, horses, cattle, sheep, pigs, dogs, cats, rats and mice. Particularly preferred tissues from which nucleic acid molecules which encode IL-1 Type 3 receptors may be isolated include brain, kidney and lung. Nucleic acid molecules which encode IL-1 Type 3 receptors of the present invention may be readily isolated from conventionally prepared cDNA libraries (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, NY, 1989) or from commercially obtained libraries (e.g., Stratagene, LaJolla, Calif.) utilizing the disclosure provided herein. Particularly preferred methods for obtaining isolated DNA molecules which encode IL-1 Type 3 receptors of the present invention are described in more detail below in Example 1 (see also Sequence I.D. Nos. 1 and 3).

As noted above, within particularly preferred embodiments of the invention, isolated nucleic acid molecules are provided which encode human IL-1 Type 3 receptors. Briefly, such nucleic acid molecules may be readily obtained by probing a human cDNA library either with a specific sequence as described below in Example 1, or with a rat sequence (e.g., Sequence I.D. Nos. 2 or 4) under conditions of high stringency (e.g., 50% formamide, 5 x SSC, 5x Denharts, 0.1% SDS, 100 ug/ml salmon sperm DNA, at 42°C for 12 hours). This may be followed by extensive washing with 2x SSC containing 0.2% SDS at 50°C. Suitable cDNA libraries may be obtained from commercial sources (e.g., Stratagene, LaJolla, Calif.; or Clontech, Palo Alto, Calif., or prepared utilizing standard techniques (see, e.g., Sambrook et al., *supra*).

PRODUCTION OF RECOMBINANT IL-1 TYPE 3 RECEPTORS

As noted above, the present invention also provides recombinant expression vectors which include synthetic or cDNA-derived DNA fragments encoding IL-1 Type 3 receptors or substantially similar proteins, which are operably linked to suitable transcriptional or translation regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and, within preferred embodiments, sequences which control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are

5 operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame.

10 Expression vectors may also contain DNA sequences necessary to direct the secretion of a polypeptide of interest. Such DNA sequences may include at least one secretory signal sequence. Representative secretory signals include the alpha factor signal sequence (pre-pro sequence; Kurjan and Herskowitz, *Cell* 30:933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, EP 116,201), the PHOS signal sequence (Beck et al., WO 86/00637), the BARI secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2 signal sequence (Carlson et al., *Mol. Cell. Biol.* 3:439-447, 1983), the α -1-antitrypsin signal sequence (Kurachi et al., *Proc. Natl. Acad. Sci. USA* 78:6826-6830, 1981), the β -2 plasmin inhibitor signal sequence (Tone et al., *J. Biochem. (Tokyo)* 102:1033-1042, 1987), the tissue plasminogen activator signal sequence (Pennica et al., *Nature* 301:214-221, 1983), the *E. coli* *PhoA* signal sequence (Yuan et al., *J. Biol. Chem.* 265:13528-13552, 1990) or any of the bacterial signal sequences reviewed, for example, by Oliver (*Annu. Rev. Microbiol.* 39:615-649, 1985). Alternatively, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (*Eur. J. Biochem.* 133:17-21, 1983; *J. Mol. Biol.* 184:99-105, 1985; *Nuc. Acids Res.* 14:4683-4690, 1986).

25 For expression, a nucleic acid molecule encoding a IL-1 Type 3 receptor is inserted into a suitable expression vector, which in turn is used to transform or transfect appropriate host cells for expression. Host cells for use in practicing the present invention include mammalian, avian, plant, insect, bacterial and fungal cells. Preferred eukaryotic cells include cultured mammalian cell lines (e.g., rodent or human cell lines) and fungal cells, including species of yeast (e.g., *Saccharomyces* spp., particularly *S. cerevisiae*, *Schizosaccharomyces* spp., or *Kluyveromyces* spp.) or filamentous fungi (e.g., *Aspergillus* spp., *Neurospora* spp.). Strains of the yeast *Saccharomyces cerevisiae* are particularly preferred. Methods for producing recombinant proteins in a variety of prokaryotic and eukaryotic host cells are generally known in the art (see "Gene Expression Technology," *Methods in Enzymology*, Vol. 185, Goeddel (ed.), Academic Press, San Diego, Calif., 1990; see also, "Guide to Yeast Genetics and Molecular Biology," *Methods in Enzymology*, Guthrie and Fink (eds.)

Academic Press, San Diego, Calif., 1991). In general, a host cell will be selected on the basis of its ability to produce the protein of interest at a high level or its ability to carry out at least some of the processing steps necessary for the biological activity of the protein. In this way, the number of cloned DNA sequences which must be transfected into the host cell may be minimized and overall yield of biologically active protein may be maximized.

5 Suitable yeast vectors for use in the present invention include YRp7 (Struhl et al., *Proc. Natl. Acad. Sci. USA* 76:1035-1039, 1978), YEp13 (Broach et al., *Gene* 8:121-133, 1979), POT vectors (Kawasaki et al., U.S. Patent No. 4,931,373, 10 which is incorporated by reference herein), pJDB249 and pJDB219 (Beiggs, *Nature* 275:104-108, 1978) and derivatives thereof. Such vectors will generally include a selectable marker, which may be one of any number of genes that exhibit a dominant phenotype for which a phenotypic assay exists to enable transformants to be selected. Preferred selectable markers are those that complement host cell auxotrophy, provide 15 antibiotic resistance or enable a cell to utilize specific carbon sources, and include *LEU2* (Broach et al., *ibid.*), *URA3* (Botstein et al., *Gene* 8:17, 1979), *HIS3* (Struhl et al., *ibid.*) or *POT1* (Kawasaki et al., *ibid.*). Another suitable selectable marker is the CAT gene, which confers chloramphenicol resistance on yeast cells.

20 Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman et al., *J. Biol. Chem.* 255:12073-12080, 1980; Alber and Kawasaki, *J. Mol. Appl. Genet.* 1:419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young et al., in *Genetic Engineering of Microorganisms for Chemicals*, Hollaender et al. (eds.), p. 355, Plenum, New York, 1982; Ammerer, *Meth. Enzymol.* 101:192-201, 1983). In this regard, particularly 25 preferred promoters are the *TP11* promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the *ADH2-4c* promoter (Russell et al., *Nature* 304:652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 07/784,653, which is incorporated herein by reference). The expression units may also include a transcriptional terminator, such as the *TP11* terminator (Alber and Kawasaki, *ibid.*).

30 In addition to yeast, proteins of the present invention can be expressed in filamentous fungi, for example, strains of the fungi *Aspergillus* (McKnight et al., U.S. Patent No. 4,935,349, which is incorporated herein by reference). Examples of useful promoters include those derived from *Aspergillus nidulans* glycolytic genes, such as the *ADH3* promoter (McKnight et al., *EMBO J.* 4:2093-2099, 1985) and the *tpi4* promoter. 35 An example of a suitable terminator is the *ADH3* terminator (McKnight et al., *ibid.*, 1985). The expression units utilizing such components are cloned into vectors that are capable of insertion into the chromosomal DNA of *Aspergillus*.

Techniques for transforming fungi are well known in the literature, and have been described, for instance, by Beggs (*ibid.*), Hinnen et al. (*Proc. Natl. Acad. Sci. USA* 75:1929-1933, 1978), Yelton et al. (*Proc. Natl. Acad. Sci. USA* 81:1740-1747, 1984), and Russell (*Nature* 301:167-169, 1983). The genotype of the host cell will generally contain a genetic defect that is complemented by the selectable marker present on the expression vector. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art. To optimize production of the heterologous proteins in yeast, for example, it is preferred that the host strain carries a mutation, such as the yeast *pep4* mutation (Jones, *Genetics* 85:23-33, 1977), which results in reduced proteolytic activity.

In addition to fungal cells, cultured mammalian cells may be used as host cells within the present invention. Preferred cultured mammalian cells for use in the present invention include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), and 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Viro.* 36:59-72, 1977) cell lines. A preferred BHK cell line is the BHK 570 cell line (deposited with the American Type Culture Collection under accession number CRL 10314). In addition, a number of other mammalian cell lines may be used within the present invention, including Rat Hep I (ATCC No. CRL 1600), Rat Hep II (ATCC No. CRL 1548), TCMK (ATCC No. CCL 139), Human lung (ATCC No. CCL 75.1), Human hepatoma (ATCC No. HTB-52), Hep G2 (ATCC No. HB 8065), Mouse liver (ATCC No. CCL 29.1), NCTC 1469 (ATCC No. CCL 9.1), SP2/0-Ag14 (ATCC No. 1581), HIT-T15 (ATCC No. CRL 1777), Ltk⁻ (ATCC) No. CCL 1.3) and RINm 5AHT₂B (Orskov and Nielson, *FEBS* 229(1):175-178, 1988).

Mammalian expression vectors for use in carrying out the present invention should include a promoter capable of directing the transcription of a cloned gene or cDNA. Preferred promoters include viral promoters and cellular promoters. Viral promoters include the immediate early cytomegalovirus promoter (Boshart et al., *Cell* 41:521-530, 1985) and the SV40 promoter (Subramani et al., *Mol. Cell. Biol.* 1:854-864, 1981). Cellular promoters include the mouse metallothionein-1 promoter (Palmiter et al., U.S. Patent No. 4,579,821), a mouse V_J promoter (Bergman et al., *Proc. Natl. Acad. Sci. USA* 81:7041-7045, 1983; Grant et al., *Nuc. Acids Res.* 15:5496, 1987) and a mouse V_H promoter (Loh et al., *Cell* 33:85-93, 1983). A particularly preferred promoter is the major late promoter from Adenovirus 2 (Kaufman and Sharp, *Mol. Cell. Biol.* 2:1304-13199, 1982). Such expression vectors may also contain a set of RNA splice sites located downstream from the promoter and upstream from the DNA sequence encoding the peptide or protein of interest. Preferred RNA splice sites may be obtained from SV40, adenovirus and/or immunoglobulin genes. Alternatively, within

certain embodiments RNA splice sites may be located downstream from the DNA sequence encoding the peptide or protein of interest. Also contained in the expression vectors is a polyadenylation signal located downstream of the coding sequence of interest. Suitable polyadenylation signals include the early or late polyadenylation signals from SV40 (Kaufman and Sharp, *ibid.*), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., *Nuc. Acids Res.* 9:3719-3730, 1981). The expression vectors may include a noncoding viral leader sequence, such as the Adenovirus 2 tripartite leader, located between the promoter and the RNA splice sites. Preferred vectors may also include 5 enhancer sequences, such as the SV40 enhancer and the mouse I enhancer (Gillies, *Cell* 33:717-728, 1983). Expression vectors may also include sequences encoding the adenovirus VA RNAs. Suitable vectors can be obtained from commercial sources (e.g., Invitrogen, San Diego, CA; Stratagene, La Jolla, CA).

Cloned DNA sequences may be introduced into cultured mammalian cells 15 by, for example, calcium phosphate-mediated transfection (Wigler et al., *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann et al., *EMBO J.* 1:841-845, 1982), or DEAE-dextran mediated transfection (Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987), which are incorporated 20 herein by reference. To identify cells that have stably integrated the cloned DNA, a selectable marker is generally introduced into the cells along with the gene or cDNA of interest. Preferred selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. Preferred amplifiable 25 selectable markers are the DHFR gene and the neomycin resistance gene. Selectable markers are reviewed by Thilly (*Mammalian Cell Technology*, Butterworth Publishers, Stoneham, MA, which is incorporated herein by reference). The choice of selectable markers is well within the level of ordinary skill in the art.

Selectable markers may be introduced into the cell on a separate vector 30 at the same time as the IL-1 Type 3 receptor sequence, or they may be introduced on the same vector. If on the same vector, the selectable marker and the IL-1 Type 3 receptor sequence may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, Levinson and Simonsen, U.S. Patent No. 4,713,339). It may 35 also be advantageous to add additional DNA, known as "carrier DNA" to the mixture which is introduced into the cells.

Transfected mammalian cells are allowed to grow for a period of time, typically 1-2 days, to begin expressing the DNA sequence(s) of interest. Drug selection is then applied to select for growth of cells that are expressing the selectable marker in a stable fashion. For cells that have been transfected with an amplifiable selectable marker 5 the drug concentration may be increased in a stepwise manner to select for increased copy number of the cloned sequences, thereby increasing expression levels. Cells expressing the introduced sequences are selected and screened for production of the protein of interest in the desired form or at the desired level. Cells which satisfy these criteria may then be cloned and scaled up for production.

Preferred prokaryotic host cells for use in carrying out the present invention are strains of the bacteria *Escherichia coli*, although *Bacillus* and other genera are also useful. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1982; or Sambrook 10 et al., *supra*). Vectors used for expressing cloned DNA sequences in bacterial hosts will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter that functions in the host cell. Appropriate promoters include the trp (Nichols and Yanofsky, *Meth. Enzymol.* 101:155-164, 1983), lac (Casadaban et al., *J. Bacteriol.* 143:971-980, 1980), and phage k (Queen, *J. Mol. Appl. Genet.* 2:1-10, 1983) promoter 15 systems. Plasmids useful for transforming bacteria include pBR322 (Bolivar et al., *Gene* 2:95-113, 1977), the pUC plasmids (Messing, *Meth. Enzymol.* 101:20-78, 1983; Vieira and Messing, *Gene* 19:259-268, 1982), pCQV2 (Queen, *ibid.*), pMAL-2 (New England Biolabs, Beverly, MA) and derivatives thereof. Plasmids may contain both viral and bacterial elements.

Given the teachings provided herein, promoters, terminators and methods 20 for introducing expression vectors encoding IL-1 Type 3 receptors of the present invention into plant, avian and insect cells would be evident to those of skill in the art. The use of baculoviruses, for example, as vectors for expressing heterologous DNA sequences in insect cells has been reviewed by Atkinson et al. (*Pestic. Sci.* 28:215- 25 224, 1990). In addition, the use of *Agrobacterium rhizogenes* as vectors for expressing genes in plant cells has been reviewed by Sinkar et al. (*J. Biosci. (Bangalore)* 11:47-58, 1987).

Host cells containing DNA molecules of the present invention are then 30 cultured to express a DNA molecule encoding a IL-1 Type 3 receptor. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the chosen host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids,

vitamins and minerals, as well as other components, e.g., growth factors or serum, that may be required by the particular host cells. The growth medium will generally select for cells containing the DNA molecules by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct.

Suitable growth conditions for yeast cells, for example, include culturing in a chemically defined medium, comprising a nitrogen source, which may be a non-amino acid nitrogen source or a yeast extract, inorganic salts, vitamins and essential amino acid supplements at a temperature between 4°C and 37°C, with 30°C being particularly preferred. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, more preferably pH 5-6. Methods for maintaining a stable pH include buffering and constant pH control. Preferred agents for pH control include sodium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Due to the tendency of yeast host cells to hyperglycosylate heterologous proteins, it may be preferable to express the IL-1 Type 3 receptors of the present invention in yeast cells having a defect in a gene required for asparagine-linked glycosylation. Such cells are preferably grown in a medium containing an osmotic stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium and growth conditions appropriate for the particular cell line used is within the level of ordinary skill in the art.

IL-1 Type 3 receptors may also be expressed in non-human transgenic animals, particularly transgenic warm-blooded animals. Methods for producing transgenic animals, including mice, rats, rabbits, sheep and pigs, are known in the art and are disclosed, for example, by Hammer et al. (*Nature* 315:680-683, 1985), Palmiter et al. (*Science* 222:809-814, 1983), Brinster et al. (*Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985), Palmiter and Brinster (*Cell* 41:343-345, 1985) and U.S. Patent No. 4,736,866, which are incorporated herein by reference. Briefly, an expression unit, including a DNA sequence to be expressed together with appropriately positioned expression control sequences, is introduced into pronuclei of fertilized eggs. Introduction of DNA is commonly done by microinjection. Integration of the injected DNA is detected by blot analysis of DNA from tissue samples, typically samples of tail tissue. It is generally preferred that the introduced DNA be incorporated into the germ line of the animal so that it is passed on to the animal's progeny.

Within particularly preferred embodiments of the invention, "knockout" animals may be developed from embryonic stem cells through the use of homologous

recombination (Capecci, *Science* 244:1288-1292, 1989) or antisense oligonucleotide (Stein and Chen, *Science* 261(5124):1004-1012, 1993; Milligan et al., *Semin. Conc. Biol.* 3(6):391-398, 1992).

5 Within a preferred embodiment of the invention, a transgenic animal, such as a mouse, is developed by targeting a mutation to disrupt a IL-1 Type 3 receptor sequence (see Mansour et al., "Disruption of the proto-oncogene *int-2* in mouse embryo-derived stem cells: A general strategy for targeting mutations to non-selectable genes," *Nature* 336:348-352, 1988). Such animals may readily be utilized as a model to study the role of the IL-1 Type 3 receptor in metabolism.

10

SOLUBLE IL-1 TYPE 3 RECEPTORS AND RECEPTOR PEPTIDES

As noted above, the present invention also provides soluble IL-1 Type 3 receptors and receptor peptides. Within the context of the present invention, IL-1 Type 3 receptor peptides should be understood to include portions of a IL-1 Type 3 receptor or derivatives thereof discussed above, which do not contain transmembrane domains, and which are at least 8, and more preferably 10 or greater amino acids in length. Briefly, the structure of the IL-1 Type 3 receptor as well as putative transmembrane domains may be predicted from the primary translation products using the hydrophobicity plot function of, for example, PROTEAN (DNA STAR, Madison, WI), 15 or according to the methods described by Kyte and Doolittle (*J. Mol. Biol.* 157:105-132, 1982). While not wishing to be bound by a graphical representation, based upon this hydrophobicity analysis, IL-1 Type 3 receptors are believed to have the general structure shown in Figure 1. In particular, these receptors are believed to comprise an extracellular amino-terminal domain, a transmembrane domain, and an intracellular 20 domain.

25 Within one aspect of the invention, isolated IL-1 Type 3 receptor peptides are provided comprising the extracellular amino-terminal domain of a IL-1 Type 3 receptor. Within a preferred embodiment, an isolated IL-1 Type 3 receptor peptide is provided comprising the sequence of amino acids shown in Sequence 1D. 30 No. 2, from amino acid number 1 to amino acid number 336. Within other embodiments, isolated IL-1 Type 3 receptor peptides are provided comprising the sequence of amino acids shown in Sequence 1D. No. 4, from amino acid number 1 to amino acid number 338.

35 IL-1 Type 3 receptor peptides may be prepared by, among other methods, culturing suitable host/vector systems to produce the recombinant translation products of the present invention. Supernatants from such cell lines may then be treated by a variety of purification procedures in order to isolate the IL-1 Type 3 receptor

peptide. For example, the supernatant may be first concentrated using commercially available protein concentration filters, such as an Amicon or Millipore Pellicon ultrafiltration unit. Following concentration, the concentrate may be applied to a suitable purification matrix such as, for example, IL-1 or an anti-IL-1 Type 3 receptor antibody bound to a suitable support. Alternatively, anion or cation exchange resins may be employed in order to purify the receptor or peptide. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps may be employed to further purify the IL-1 Type 3 receptor peptide.

10 Alternatively, IL-1 Type 3 receptor peptides may also be prepared utilizing standard polypeptide synthesis protocols, and purified utilizing the above-described procedures.

15 A IL-1 Type 3 receptor peptide is deemed to be "isolated" or purified within the context of the present invention, if only a single band is detected subsequent to SDS-polyacrylamide gel analysis followed by staining with Coomassie Brilliant Blue.

15

ANTIBODIES TO IL-1 TYPE 3 RECEPTORS

Within one aspect of the present invention, IL-1 Type 3 receptors, including derivatives thereof, as well as portions or fragments of these proteins such as the IL-1 Type 3 receptor peptides discussed above, may be utilized to prepare antibodies 20 which specifically bind to IL-1 Type 3 receptors. Within the context of the present invention the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, fragments thereof such as $F(ab')_2$ and Fab fragments, as well as recombinantly produced binding partners. These binding partners incorporate the variable regions from a gene which encodes a specifically binding monoclonal antibody. Antibodies are defined to be 25 specifically binding if they bind to the IL-1 Type 3 receptor with a K_A of greater than or equal to $10^7 M^{-1}$ and preferably greater than or equal to $10^8 M^{-1}$, and bind to IL-1 Type I or Type II receptors with an affinity of less than $K_A 10^7 M^{-1}$, and preferably less than $10^5 M^{-1}$ or $10^3 M^{-1}$. The affinity of a monoclonal antibody or binding partner may be readily determined by one of ordinary skill in the art (see Scatchard, *Ann. N.Y. Acad. Sci. 51*:660-672, 1949).

30 Polyclonal antibodies may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats. Briefly, the IL-1 Type 3 receptor is utilized to immunize the animal through intraperitoneal, intramuscular, intraocular, or subcutaneous 35 injections. The immunogenicity of a IL-1 Type 3 receptor or IL-1 Type 3 receptor peptide may be increased through the use of an adjuvant such as Freund's complete or incomplete adjuvant. Following several booster immunizations, small samples of serum

are collected and tested for reactivity to the IL-1 Type 3 receptor. A variety of assays may be utilized in order to detect antibodies which specifically bind to a IL-1 Type 3 receptor. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988. Representative 5 examples of such assays include: Countercurrent Immuno-Electrophoresis (CIEP), Radioimmunoassays, Radioimmunoprecipitations, Enzyme-Linked Immuno-Sorbent Assays (ELISA), Dot Blot assays, Inhibition or Competition assays, and sandwich assays (see U.S. Patent Nos. 4,376,110 and 4,486,530; see also *Antibodies: A Laboratory Manual, supra*). Particularly preferred polyclonal antisera will give a signal 10 that is at least three times greater than background. Once the titer of the animal has reached a plateau in terms of its reactivity to the IL-1 Type 3 receptor, larger quantities of polyclonal antisera may be readily obtained either by weekly bleedings, or by exsanguinating the animal.

Monoclonal antibodies may also be readily generated using well-known 15 techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; see also *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, within one embodiment a subject animal such as a rat or mouse is 20 injected with a form of IL-1 Type 3 receptor suitable for generating an immune response against the IL-1 Type 3 receptor. Representative examples of suitable forms include, among others, cells which express the IL-1 Type 3 receptor, or peptides which are based upon the IL-1 Type 3 receptor sequence. Additionally, many techniques are known in the art for increasing the resultant immune response, for example, by coupling the 25 receptor or receptor peptides to another protein such as ovalbumin or keyhole limpet hemocyanin (KLH), or through the use of adjuvants such as Freund's complete or incomplete adjuvant. The initial immunization may be through intraperitoneal, intramuscular, intraocular, or subcutaneous routes.

Between one and three weeks after the initial immunization the animal 30 may be reimmunized with another booster immunization. The animal may then be tested and the serum tested for binding to the IL-1 Type 3 receptor using assays as described above. Additional immunizations may also be accomplished until the animal has plateaued in its reactivity to the IL-1 Type 3 receptor. The animal may then be given a final boost of IL-1 Type 3 receptor or IL-1 Type 3 receptor peptide, and three 35 to four days later sacrificed. At this time, the spleen and lymph nodes may be harvested and disrupted into a single cell suspension by passing the organs through a mesh screen or by rupturing the spleen or lymph node membranes which encapsidate the cells.

Within one embodiment the red cells are subsequently lysed by the addition of a hypotonic solution, followed by immediate return to isotonicity.

Within another embodiment, suitable cells for preparing monoclonal antibodies are obtained through the use of *in vitro* immunization techniques. Briefly, an

5 animal is sacrificed, and the spleen and lymph node cells are removed as described above. A single cell suspension is prepared, and the cells are placed into a culture containing a form of the IL-1 Type 3 receptor that is suitable for generating an immune response as described above. Subsequently, the lymphocytes are harvested and fused as described below.

10 Cells which are obtained through the use of *in vitro* immunization or from an immunized animal as described above may be immortalized by transfection with a virus such as the Epstein-Barr virus (EBV) (see Glasky and Reading, *Hybridoma* 8(4):377-389, 1989). Alternatively, within a preferred embodiment, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in
15 order to create a "hybridoma" which secretes monoclonal antibodies. Suitable myeloma lines are preferably defective in the construction or expression of antibodies, and are additionally syngeneic with the cells from the immunized animal. Many such myeloma cell lines are well known in the art and may be obtained from sources such as the American Type Culture Collection (ATCC), Rockville, Maryland (see *Catalogue of Cell Lines & Hybridomas*, 6th ed., ATCC, 1988). Representative myeloma lines include: for humans, UC 729-6 (ATCC No. CRL 8061), MC/CAR-Z2 (ATCC No. CRL 8147), and SKO-007 (ATCC No. CRL 8033); for mice, SP2/0-Ag14 (ATCC No. CRL 1581), and P3X63Ag8 (ATCC No. TIB 9); and for rats, Y3-Ag1.2.3 (ATCC No. CRL 1631), and YB2/0 (ATCC No. CRL 1662). Particularly preferred fusion lines include NS-1 (ATCC
20 No. TIB 18) and P3X63 - Ag 8.653 (ATCC No. CRL 1580), which may be utilized for fusions with either mouse, rat, or human cell lines. Fusion between the myeloma cell line and the cells from the immunized animal may be accomplished by a variety of methods, including the use of polyethylene glycol (PEG) (see *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988) or
25 electrofusion (see Zimmerman and Vienken, *J. Membrane Biol.* 67:165-182, 1982).

30

Following the fusion, the cells are placed into culture plates containing a suitable medium, such as RPMI 1640 or DMEM (Dulbecco's Modified Eagles Medium) (JRH Biosciences, Lenexa, KS). The medium may also contain additional ingredients, such as Fetal Bovine Serum ("FBS," i.e., from Hyclone, Logan, Utah, or JRH Bi sciences), thymocytes which were harvested from a baby animal of the same species as was used for immunization, or agar to solidify the medium. Additionally, the medium should contain a reagent which selectively allows for the growth of fused spleen and

myel ma cells. Particularly preferred is the use of HAT (hypoxanthine, aminopterin, and thymidine) (Sigma Chemical Co., St. Louis, MO). After about seven days, the resulting fused cells or hybridomas may be screened in order to determine the presence of antibodies which recognize the IL-1 Type 3 receptor. Following several clonal 5 dilutions and reassays, a hybridoma producing antibodies which bind to IL-1 Type 3 receptor may be isolated.

Other techniques may also be utilized to construct monoclonal antibodies (see Huse et al., "Generation of a Large Combinational Library of the Immunoglobulin Repertoire in Phage Lambda," *Science* 246:1275-1281, December 1989; see also Sastry et al., "Cloning of the Immunological Repertoire in *Escherichia coli* for Generation of 10 Monoclonal Catalytic Antibodies: Construction of a Heavy Chain Variable Region-Specific cDNA Library," *Proc. Natl. Acad. Sci. USA* 86:5728-5732, August 1989; see also Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas," *Strategies in Molecular Biology* 3:1-9, January 1990; these 15 references describe a commercial system available from Stratocyte, La Jolla, California, which enables the production of antibodies through recombinant techniques). Briefly, mRNA is isolated from a B cell population and utilized to create heavy and light chain immunoglobulin cDNA expression libraries in the kIMMUNOZAP(H) and kIMMUNOZAP(L) vectors. These vectors may be screened individually or co- 20 expressed to form Fab fragments or antibodies (see Huse et al., *supra*; see also Sastry et al., *supra*). Positive plaques may subsequently be converted to a non-lytic plasmid which allows high level expression of monoclonal antibody fragments from *E. coli*.

Similarly, binding partners may also be constructed utilizing recombinant 25 DNA techniques to incorporate the variable regions of a gene which encodes a specifically binding antibody. The construction of these proteins may be readily accomplished by one of ordinary skill in the art (see Larrick et al., "Polymerase Chain Reaction Using Mixed Primers: Cloning of Human Monoclonal Antibody Variable Region Genes From Single Hybridoma Cells," *Biotechnology* 7:934-938, September 1989; Riechmann et al., "Reshaping Human Antibodies for Therapy," *Nature* 332:323-30 327, 1988; Roberts et al., "Generation of an Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering," *Nature* 328:731-734, 1987; Verhoeyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity," *Science* 239:1534-1536, 1988; Chaudhary et al., "A Recombinant Immunotoxin Consisting f Two Antibody Variable Domains Fused to *Pseudomonas* Exotoxin," 35 *Nature* 339:394-397, 1989; see also, U.S. Patent No. 5,132,405 entitled "Biosynthetic Antibody Binding Sites"), given the disclosure provided herein. Briefly, within one embodiment, DNA molecules encoding IL-1 Type 3 receptor-specific antigen binding

domains are amplified from hybridomas which produce a specifically binding monoclonal antibody, and inserted directly into the genome of a cell which produces human antibodies (see Verhoeven et al., *supra*; see also Reichmann et al., *supra*). This technique allows the antigen-binding site of a specifically binding mouse or rat 5 monoclonal antibody to be transferred into a human antibody. Such antibodies are preferable for therapeutic use in humans because they are not as antigenic as rat or mouse antibodies.

Alternatively, the antigen-binding sites (variable region) may be either 10 linked to, or inserted into, another completely different protein (see Chaudhary et al., *supra*), resulting in a new protein with antigen-binding sites of the antibody as well as the functional activity of the completely different protein. As one of ordinary skill in the art will recognize, the antigen-binding sites or IL-1 Type 3 receptor binding domain of the antibody may be found in the variable region of the antibody. Furthermore, DNA sequences which encode smaller portions of the antibody or variable regions which 15 specifically bind to mammalian IL-1 Type 3 receptor may also be utilized within the context of the present invention. These portions may be readily tested for binding specificity to the IL-1 Type 3 receptor utilizing assays described below.

Within a preferred embodiment, genes which encode the variable region 20 from a hybridoma producing a monoclonal antibody of interest are amplified using oligonucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. Stratacyte (La Jolla, CA) sells primers for mouse and human variable regions including, among others, primers for $V_{H\alpha}$, $V_{H\beta}$, $V_{H\gamma}$, $V_{H\delta}$, C_{H1} , V_L and C_L regions. These primers may be utilized to amplify heavy or light chain variable regions, which 25 may then be inserted into vectors such as IMMUNOZAP[®](H) or IMMUNOZAP[®](L) (Stratacyte), respectively. These vectors may then be introduced into *E. coli* for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced (see Bird et al., *Science* 242:423-426, 1988).

30 Other "antibodies" which may also be prepared utilizing the disclosure provided herein, and thus which are also deemed to fall within the scope of the present invention include humanized antibodies (e.g., U.S. Patent No. 4,816,567 and WO 94/10332), micobodies (e.g., WO 94/09817) and transgenic antibodies (e.g., GB 2 272 440).

35 Once suitable antibodies have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (see *Antibodies: A Laboratory Manual*, *supra*). Suitable techniques include peptide or

protein affinity columns, HPLC or RP-HPLC, purification on protein A or protein G columns, or any combination of these techniques. Within the context of the present invention, the term "isolated" as used to define antibodies or binding partners means "substantially free of other blood components."

5 Antibodies of the present invention have many uses. For example, antibodies may be utilized in flow cytometry to sort IL-1 Type 3 receptor-bearing cells, or to histochemically stain IL-1 Type 3 receptor-bearing tissues. Briefly, in order to detect IL-1 Type 3 receptors on cells, the cells (or tissue) are incubated with a labeled antibody which specifically binds to IL-1 Type 3 receptors, followed by detection of the
10 presence of bound antibody. These steps may also be accomplished with additional steps such as washings to remove unbound antibody. Representative examples of suitable labels, as well as methods for conjugating or coupling antibodies to such labels are described in more detail below.

15 In addition, purified antibodies may also be utilized therapeutically to block the binding of IL-1 or other IL-1 Type 3 receptor substrates to the IL-1 Type 3 receptor *in vitro* or *in vivo*. As noted above, a variety of assays may be utilized to detect antibodies which block or inhibit the binding of IL-1 to the IL-1 Type 3 receptor, including *inter alia*, inhibition and competition assays noted above. Within one embodiment, monoclonal antibodies (prepared as described above) are assayed for
20 binding to the IL-1 Type 3 receptor in the absence of IL-1, as well as in the presence of varying concentrations of IL-1. Blocking antibodies are identified as those which, for example, bind to IL-1 Type 3 receptors and, in the presence of IL-1, block or inhibit the binding of IL-1 to the IL-1 Type 3 receptor.

25 Antibodies of the present invention may also be coupled or conjugated to a variety of other compounds (or labels) for either diagnostic or therapeutic use. Such compounds include, for example, toxic molecules, molecules which are nontoxic but which become toxic upon exposure to a second compound, and radionuclides. Representative examples of such molecules are described in more detail below.

30 Antibodies which are to be utilized therapeutically are preferably provided in a therapeutic composition comprising the antibody or binding partner and a physiologically acceptable carrier or diluent. Suitable carriers or diluents include, among others, neutral buffered saline or saline, and may also include additional excipients or stabilizers such as buffers, sugars such as glucose, sucrose, or dextrose, chelating agents such as EDTA, and various preservatives.

LABELS

The nucleic acid molecules, antibodies, and IL-1 Type 3 receptors (including sIL-1 3R) of the present invention may be labeled or conjugated (either through covalent or non-covalent means) to a variety of labels or other molecules, 5 including for example, fluorescent markers, enzyme markers, toxic molecules, molecules which are nontoxic but which become toxic upon exposure to a second compound, and radionuclides.

Representative examples of fluorescent labels suitable for use within the present invention include, for example, Fluorescein Isothiocyanate (FITC), Rhodamine, 10 Texas Red, Luciferase and Phycoerythrin (PE). Particularly preferred for use in flow cytometry is FITC which may be conjugated to purified antibody according to the method of Keltkamp in "Conjugation of Fluorescein Isothiocyanate to Antibodies. I. Experiments on the Conditions of Conjugation," *Immunology* 18:865-873, 1970. (See also Keltkamp, "Conjugation of Fluorescein Isothiocyanate to Antibodies. II. A 15 Reproducible Method," *Immunology* 18:875-881, 1970; and Goding, "Conjugation of Antibodies with Fluorochromes: Modification to the Standard Methods," *J. Immunol. Methods* 13:215-226, 1970.) For histochemical staining, HRP, which is preferred, may be conjugated to the purified antibody according to the method of Nakane and Kawaoi ("Peroxidase-Labeled Antibody: A New Method of Conjugation," *J. Histochem. Cytochem.* 22:1084-1091, 1974; see also, Tijssen and Kurstak, "Highly Efficient and Simple Methods for Preparation of Peroxidase and Active Peroxidase Antibody Conjugates for Enzyme Immunoassays," *Anal. Biochem.* 136:451-457, 1984).

Representative examples of enzyme markers or labels include alkaline phosphatase, horse radish peroxidase, and β -galactosidase. Representative examples of 25 toxic molecules include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, pokeweed antiviral protein, tritin, Shigella toxin, and *Pseudomonas* exotoxin A. Representative examples of molecules which are nontoxic, but which become toxic upon exposure to a second compound include thymidine kinases such as HSVTK and VZVTK. Representative examples of radionuclides include Cu-64, Ga-67, Ga-68, Zr-89, Ru-97, 30 Tc-99m, Rh-105, Pd-109, In-111, I-123, I-125, I-131, Re-186, Re-188, Au-198, Au-199, Pb-203, At-211, Pb-212 and Bi-212.

As will be evident to one of skill in the art given the disclosure provided herein, the above described nucleic acid molecules, antibodies, and IL-1 Type 3 receptors may also be labeled with other molecules such as colloidal gold, as well either 35 member of a high affinity binding pair (e.g., avidin-biotin).

DIAGNOSTIC USE OF IL-1 TYPE 3 RECEPTOR SEQUENCES

Within another aspect of the present invention, probes and primers are provided for detecting IL-1 Type 3 receptors. Within one embodiment of the invention, probes are provided which are capable of hybridizing to IL-1 Type 3 receptor DNA or RNA. For purposes of the present invention, probes are "capable of hybridizing" to IL-1 Type 3 receptor DNA if they hybridize to Sequence I.D. Nos. 1 or 3 under conditions of moderate or high stringency (see Sambrook et al., *supra*); but not to IL-1 Type I or Type II receptor nucleic acid sequences. Preferably, the probe may be utilized to hybridize to suitable nucleotide sequences in the presence of 50% formamide, 5x SSPE, 10x Denhardt's, 0.1% SDS and 100 ug/ml Salmon Sperm DNA at 42°C, followed by a first wash with 2x SSC at 42°C, and a second wash with 0.2x SSC at 55 to 60°C.

Probes of the present invention may be composed of either deoxyribonucleic acids (DNA) ribonucleic acids (RNA), nucleic acid analogues, or any combination of these, and may be as few as about 12 nucleotides in length, usually about 15 14 to 18 nucleotides in length, and possibly as large as the entire sequence of the IL-1 Type 3 receptor. Selection of probe size is somewhat dependent upon the use of the probe. For example, in order to determine the presence of various polymorphic forms of the IL-1 Type 3 receptor within an individual, a probe comprising virtually the entire length of the IL-1 Type 3 receptor coding sequence is preferred. IL-1 Type 3 receptor probes may be utilized to identify polymorphisms linked to the IL-1 Type 3 receptor gene (see, for example, Weber, *Genomics* 7:524-530, 1990; and Weber and May, *Amer. J. Hum. Gen.* 44:388-396, 1989). Such polymorphisms may be associated with inherited diseases such as diabetes.

Probes may be constructed and labeled using techniques which are well known in the art. Shorter probes of, for example, 12 or 14 bases may be generated synthetically. Longer probes of about 75 bases to less than 1.5 kb are preferably generated by, for example, PCR amplification in the presence of labeled precursors such as ³²P-dCTP, digoxigenin-dUTP, or biotin-dATP. Probes of more than 1.5 kb are generally most easily amplified by transfecting a cell with a plasmid containing the relevant probe, growing the transfected cell into large quantities, and purifying the relevant sequence from the transfected cells (see Sambrook et al., *supra*).

Probes may be labeled by a variety of markers including, for example, radioactive markers, fluorescent markers, enzymatic markers, and chromogenic markers. The use of ³²P is particularly preferred for marking or labeling a particular probe.

Probes of the present invention may also be utilized to detect the presence of a IL-1 Type 3 receptor mRNA or DNA within a sample. However, if IL-1 Type 3 receptors are present in only a limited number, or if it is desired to detect a

selected mutant sequence which is present in only a limited number, or if it is desired to clone a IL-1 Type 3 receptor from a selected warm-blooded animal, then it may be beneficial to amplify the relevant sequence such that it may be more readily detected or obtained.

5 A variety of methods may be utilized in order to amplify a selected sequence, including, for example, RNA amplification (see Lizardi et al., *Bio/Technology* 6:1197-1202, 1988; Kramer et al., *Nature* 339:401-402, 1989; Lomeli et al., *Clinical Chem.* 35(9):1826-1831, 1989; U.S. Patent No. 4,786,600), and DNA amplification utilizing Polymerase Chain Reaction ("PCR") (see U.S. Patent Nos. 4,683,195, 10 4,683,202, and 4,800,159) (see also, U.S. Patent Nos. 4,876,187, and 5,011,769, which describe an alternative detection/amplification system comprising the use of scissile linkages).

15 Within a particularly preferred embodiment, PCR amplification is utilized to detect or obtain a IL-1 Type 3 receptor DNA. Briefly, as described in greater detail below, a DNA sample is denatured at 95°C in order to generate single stranded DNA. Specific primers, as discussed below, are then annealed at 37°C to 70°C, depending on the proportion of AT/GC in the primers. The primers are extended at 72°C with Taq polymerase in order to generate the opposite strand to the template. These steps constitute one cycle, which may be repeated in order to amplify the selected sequence.

20 Primers for the amplification of a selected sequence should be selected from sequences which are highly specific and form stable duplexes with the target sequence. The primers should also be non-complementary, especially at the 3' end, should not form dimers with themselves or other primers, and should not form secondary structures or duplexes with other regions of DNA. In general, primers of about 18 to 20 nucleotides are preferred, and may be easily synthesized using techniques well known in the art.

PHARMACEUTICAL COMPOSITIONS AND THERAPEUTIC USES

30 As noted above, the present invention provides pharmaceutical compositions, as well as methods for using the same (for either prophylactic or therapeutic use). Briefly, the pharmaceutical compositions of the present invention may comprise an IL-1 3R, sIL-1 3R, antibody which is capable of specifically binding IL-1 3R, IL-1 3R antagonists or agonists, in combination with a pharmaceutically acceptable carrier, diluent, or excipient. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like, carbohydrates such as glucose, mannose, sucrose or dextrose, proteins, polypeptides or amino acids, 35 antioxidants, chelating agents such as EDTA or glutathione, and preservatives.

5 Compositions of the present invention may be formulated for the manner of administration indicated, including for example, for oral, nasal, venous, vaginal or rectal administration. Within other embodiments, the compositions may be administered as part of a sustained release implant (e.g., intra-articularly). Within yet other embodiments, the compositions may be formulated as a lyophilizate, utilizing appropriate excipients which provide stability as a lyophilizate, and subsequent to rehydration.

10 Pharmaceutical compositions of the present invention may be utilized in order to treat a wide variety of diseases including, for example, immune-associated diseases such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, myasthenia gravis, scleritis, scleroderma, septic shock, allograft rejection, and graft 15 versus host (GVH) disease. In particular, pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). Although appropriate dosages may be determined by clinical trials, the quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease.

20 Within other aspects of the present invention, viral vectors are provided which may be utilized to treat diseases wherein either the IL-1 Type 3 receptor (or a mutant IL-1 Type 3 receptor) is over-expressed, or where no IL-1 Type 3 receptor is expressed. Briefly, within one embodiment of the invention, viral vectors are provided which direct the production of antisense IL-1 Type 3 receptor RNA, in order to prohibit the over-expression of IL-1 Type 3 receptors, or the expression of mutant IL-1 Type 3 receptors. Within another embodiment, viral vectors are provided which direct the expression of IL-1 Type 3 receptor cDNA. Viral vectors suitable for use in the present invention include, among others, recombinant vaccinia vectors (U.S. Patent Nos. 25 4,603,112 and 4,769,330), recombinant pox virus vectors (PCT Publication No. WO 89/01973), and preferably, recombinant retroviral vectors ("Recombinant Retroviruses with Amphotropic and Ectotropic Host Ranges," PCT Publication No. WO 90/02806; "Retroviral Packaging Cell Lines and Processes of Using Same," PCT Publication No. WO 89/07150; and "Antisense RNA for Treatment of Retroviral Disease States," PCT Publication No. WO/03451), and herpesvirus vectors (Kit, *Adv. Exp. Med. Biol.* 215:219-236, 1989; U.S. Patent No. 5,288,641).

30 35 Within various embodiments of the invention, the above-described compositions may be administered *in vivo*, or *ex vivo*. Representative routes for *in vivo* administration include intradermally ("i.d."), intracranially ("i.c."), intraperitoneally ("i.p."), intrathecally ("i.t."), intravenously ("i.v."), subcutaneously ("s.c.") or intramuscularly ("i.m.").

Within other embodiments of the invention, the vectors which contain or express nucleic acid molecules of the present invention, or even the nucleic acid molecules themselves, may be administered by a variety of alternative techniques, including for example direct DNA injection (Acsadi et al., *Nature* 352:815-818, 1991);

5 microprojectile bombardment (Williams et al., *PNAS* 88:2726-2730, 1991); liposomes (Pickering et al., *Circ.* 89(1):13-21, 1994; and Wang et al., *PNAS* 84:7851-7855, 1987); lipofection (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1989); DNA ligand (Wu et al., *J. Biol. Chem.* 264:16985-16987, 1989); administration of DNA linked to killed adenovirus (Michael et al., *J. Biol. Chem.* 268(10):6866-6869, 1993; and

10 Curiel et al., *Hum. Gene Ther.* 3(2):147-154, 1992), retrotransposons, cytofectin-mediated introduction (DMRIE-DOPE, Vical, Calif.) and transferrin-DNA complexes (Zenke).

The following examples are offered by way of illustration, and not by
15 way of limitation.

EXAMPLESEXAMPLE 1

5

ISOLATION OF INTERLEUKIN-1 TYPE 3 RECEPTOR cDNA

A. Isolation of Interleukin-1 Type 3 Receptor cDNA From a Rat Lung cDNA Library

Male Sprague-Dawley rats (Madison, WI) weighing between 175-250 gm are decapitated, and the lungs excised. Total RNA is then isolated from the lung utilizing a Promega RNaGents Total RNA Kit (catalog #Z5110, Promega, Wisc.) according to the manufacturers instructions, followed by the isolation of poly A+ RNA utilizing a Promega PolyATract kit (catalog # Z5420). A cDNA phage library is then prepared utilizing a Giga-Pack Gold library construction kit according to the manufacturer's instructions (catalog #237611, Stratagene, LaJolla, Calif.), which is in turn plated and screened essentially as described by Sambrook et al., (Molecular Cloning) with oligonucleotide (5'-CTTCAACTGC ACATACCCCTC CAGTAACAAA CGGGGCAGTG AATCTGACAT-3') (Sequence I.D. No. 6). This oligonucleotide is complementary to nucleotides 211-260 of the rat IL-1 Type 3 receptor cDNA sequence shown in Sequence I.D. No. 3.

The phage library is rescreened until a single pure phage isolate is obtained. The phage is then grown on bacterial host XL1-Blue (Stratagene, LaJolla, Calif.), and plasmid DNA is excised with ExAssist helper phage (Stratagene) in SOLR cells. The SOLR cells are then plated, and plasmid DNA is isolated and sequenced utilizing the Sanger dideoxy protocol.

A rat IL-1 Type 3 receptor cDNA sequence that may be obtained utilizing this procedure is set forth in Sequence I.D. No. 3.

B. Isolation of Interleukin-1 Type 3 Receptor cDNA From a Commercially Available Rat cDNA Library

IL-1 Type 3 receptor cDNA can also be isolated from commercially available rat cDNA libraries. For example, two million plaques from a rat phage library (Clontech, catalog # RL1048a) may be plated according to the manufacturer's instructions, and screened with oligonucleotide Sequence I.D. No. 6 essentially as described above.

A rat IL-1 Type 3 receptor cDNA sequence that may be obtained utilizing this procedure is set forth in Sequence I.D. No. 3.

C. Isolation of IL-1 Type 3 Receptor cDNA From a Human cDNA Library

IL-1 Type 3 receptor cDNA can also be isolated from commercially available human cDNA libraries. Briefly, approximately two million plaques from a 5 human phage library (Clontech, catalog # HL1158a) are plated according to the manufacturers instructions, and screened with oligonucleotide (5'-CCTCCCATAA CATCTGGGA AGTCAGTGTA ACATGGTATA AAAATTCTAG C-3') (Sequence I.D. No. 7) essentially as described above. This oligonucleotide is complementary to nucleotides 260-310 of the human IL-1 Type 3 receptor cDNA sequence shown in 10 Sequence I.D. No. 1.

The phage library is rescreened and isolated as described above. The human sequence that is obtained utilizing this procedure is approximately 89.1% identical at the nucleotide level and 89.2% identical at the amino acid level to that of the common region of the above-described rat IL-1 Type 3 receptors.

15

EXAMPLE 2

EXPRESSION OF IL-1 TYPE 3 RECEPTOR cDNA

20 A. Expression of Rat Interleukin-1 Type 3 Receptor

In order to express IL-1 Type 3 receptor cDNA, a mammalian cell expression vector (pCDM7amp) is first constructed. Briefly, pCDM7amp is a DNA 25 plasmid which contains 1) an ampicillin resistance gene that provides for selection in prokaryotic cells, 2) a bacterial origin of replication which allows propagation and amplification in host bacterial cells, 3) a CMV (cytomegalovirus) promoter which sponsors transcription in mammalian cells, 4) a multiple cloning site (MCS), which is a series of adjacent restriction sites in the DNA sequence that are useful for the insertion of appropriate DNA fragments, and 5) a SV 40 T-antigen splice and polyadenylation site.

30 pCDM7-Amp is constructed from pCDM8 (Seed, *Nature* 329:840-842, 1987; Seed and Aruffo, *Proc. Natl. Acad. Sci.* 84:3365-3369, 1987; Thomsen et al., *Cell* 63:485-493, 1990; Bernot and Auffray, *Proc. Natl. Acad. Sci.* 88:2550-2554, 1991; Han et al., *Nature* 349:697-700, 1991) by deletion of the adeno origin of replication, M13 origin of replication and sup F selection marker. An ampicillin resistance marker is then 35 added in order to facilitate selection of the plasmid.

A full-length rat IL-1 Type 3 receptor clone in pBluescriptSK- is isolated from the phage clone described above, and cut with *Eco*RV and *Hind*III, releasing two

inserts. The inserts are then isolated and ligated to pCDM7-Amp which had been similarly cut. The resulting product is used to transform *E. coli* DH5 α , and colonies are examined by restriction digests for correct orientation of the two inserts (i.e., proper formation of the IL-1 Type 3R coding sequence.)

5 COS-7 (ATCC No. CRL 1651) cells are then transfected with pCDM7-Amp containing IL-1 Type 3 receptor cDNA (10 ug DNA/10 cm plate of cells) utilizing 400 μ g/ml of DEAE-Dextran and 100 μ M chloroquine. The cells are transfected for 4 hours, then shocked with 10% DMSO for 2 minutes. The cells are then washed, and grown in DMEM containing 10% Fetal Bovine Serum for 2 days in a 24-well plate.

10

B. Expression of Human Interleukin-1 Type 3 Receptor

A full-length human IL-1 Type 3 receptor clone in pBluescriptSK- is isolated from the phage clone described above, and cut with *Nor*I and *Xba*I, releasing the insert. The insert is then isolated and ligated to pCDM7-Amp which had been similarly cut. The resulting product is used to transform *E. coli* DH5 α , from which larger quantities of plasmid DNA may be isolated.

15 COS-7 (ATCC No. CRL 1651) cells are then transfected with pCDM7-Amp containing IL-1 Type 3 receptor cDNA (10 ug DNA/10 cm plate of cells) utilizing 400 μ g/ml of DEAE-Dextran and 100 μ M chloroquine. The cells are transfected for 4 hours, then shocked with 10% DMSO for 2 minutes. The cells are then washed, and grown in DMEM containing 10% Fetal Bovine Serum for 2 days in a 24-well plate.

25

EXAMPLE 3
CONSTRUCTION AND EXPRESSION OF SOLUBLE
HUMAN INTERLEUKIN-1 TYPE 3 RECEPTOR

A. Plasmid Construction

1. Vector Preparation

30 An expression vector containing the N-terminal portion of the human IL-1 type 3 receptor, also referred to as the "soluble" form of the receptor, is constructed essentially as described below. Briefly, pCDM7amp DNA (as described above) is subjected to restriction endonuclease digestion with two enzymes, *Nor*I and *Xba*I, each of which have one recognition site in this vector, both located in the MCS. The product 35 is a linearized DNA fragment with the CMV promoter/enhancer immediately upstream of the cut site, and the polyadenylation signal downstream of the cut site.

After digestion, the cleaved vector is isolated by agarose gel electrophoresis and purified using the Gene Clean procedure (Bio 101, San Diego, CA). The vector is now ready to combine with a DNA fragment encoding the soluble human IL-1 type 3 receptors.

5

2. Insert Preparation

Into this prepared vector is ligated a DNA fragment containing the coding region of the first 336 amino acids of the human IL-1 type 3 receptor set forth in Sequence ID No. 1 (from nucleotide number 129 to nucleotide number 1136).

10

Briefly, two oligonucleotides are first synthesized for use as primers in PCR. These oligonucleotides can be synthesized on a DNA synthesizer. The first primer consists of the sequence 5'-CCTACTCGAG ATGTGGCCT TGCTGCTC-3' (Sequence ID No: 8). The first four nucleotides of this sequence serve as a spacer, and increase the efficiency of endonuclease cleavage in a subsequent reaction to be described. Nucleotides 5 through 10 encode a *Xba*I endonuclease cleavage site, and nucleotides 11 through 28 are identical to the N-terminal coding region of the human IL-type 3 receptor (nucleotides 129 to 146 in Sequence ID No: 1)).

15

The second primer consists of the sequence 5'-ATGCGCGGCC GCCTATCGAA AATCCGGAGC TGG-3' (Sequence Id No: 9). The first four nucleotides of this sequence serve as a spacer, and increase efficiency of endonuclease cleavage in a subsequent reaction to be described. Nucleotides 5 through 12 encode a *No*l endonuclease cleavage site. Nucleotides 13 through 15 encode a translation stop codon, and nucleotides 16 though 33 are complementary to the coding region of the human IL-1 type 3 receptor immediately preceding the transmembrane region (nucleotides 1133 through 1116 in Sequence ID No. 1).

20

The fragment encoding soluble human IL-1 type 3 receptor is then generated by PCR. Briefly, 100ng of each primer are combined in a 0.5ml test tube, along with 1ng of the entire human IL-1 type 3 receptor DNA sequence contained in a cloning vector, such as Bluescript (Stratagene, La Jolla, CA). Ten microliters of 10X PCR buffer, 5ul of 25mM MgCl₂, 1ul of 25mM aTTP, and 1ul of Taq polymerase/Vent polymerase (16:1 ratio) are also added to the reaction. The complete sample is then overlayed with 100ul of mineral oil to prevent evaporation, and the sample is placed in a thermocycler. Reaction conditions are: 94°C for 15 seconds, 55°C for 60 seconds, and 72°C for 60 seconds. These conditions are repeated for 25 cycles.

25

Product from the reaction is analyzed by agarose gel electrophoresis to verify the size of the fragment (1009 bp) and also to determine the approximate amount of DNA generated. The DNA is then isolated by phenol/chloroform extraction and

purified over a G-50 mini-spin column (Boehringer Mannheim, Indianapolis, IN). Approximately 10ug of the purified DNA fragment is digested with 20 units each of *Xba*I and *Nsi*I restriction endonucleases in a standard reaction to generate cohesive ends on the fragment which are compatible with the pCDM7 vector prepared as detailed above. The digested fragment is then agarose gel purified to remove impurities and contaminating DNA species.

3. Ligation

One hundred nanograms of vector DNA is combined with 100ng of insert DNA in a 1.5ml mini-tube with 1ul of 10X ligation buffer, 1ul of DNA ligase (Boehringer Mannheim), and water to a total volume of 10ul. This sample is incubated at 23°C for 2 hours.

4. Transformation

One hundred microliters of competent *E. coli* bacteria cells are combined with the ligation product and incubated on ice for 30 minutes. The sample is then incubated at 42°C for 45 seconds. One milliliter of bacterial medium (Circle Grow, Bio 101, San Diego, CA) is then added, and the sample is shaken at 37°C for 60 minutes. The sample is then plated on a bacterial growth plate containing bacterial medium and ampicillin at 100ug/ml (Fisher Scientific), and incubated for 16 hours at 37°C.

5. Construct verification

Ten colonies from the ampicillin plate are selected and grown in 1 ml of bacterial medium for 24 hours. One hundred microliters of each culture is stored by adding an equal volume of 50% glycerol solution and frozen at -70°C in mini-tubes. Plasmid DNA is then extracted from the remaining cultures by the mini-prep procedure essentially as described by Maniatis et al. (*supra*), and the recovered DNAs are analyzed by restriction digest with *Xba*I and *Nsi*I restriction endonucleases. The products of restriction digest are visualized by agarose gel electrophoresis and ethidium bromide staining. Correct plasmids will yield two bands: a vector band of approximately 3 kilobases, and an insert fragment of 1009 bases.

The frozen stock of a colony containing the correct plasmid is used to inoculate one liter of bacterial growth medium containing ampicillin (100ug/ml). The culture is shaken at 37°C for 24 hours, and plasmid DNA is isolated by a maxi-prep procedure (Promega). The portion on this plasmid coding for soluble human IL-1 type 3 receptor is analyzed by DNA sequencing (US Biochemical) in order to verify that the sequence is correct.

15 B. Transfection Procedure and Expression

COS-7 (ATCC No. CRL 1651) or L-tk⁻ cells (ATCC No. CCL 1.3) are seeded at 1×10^6 or 3×10^6 cells on 10 cm tissue culture dishes and incubated over night.

5 Cells are then transfected by a standard DEAE dextran method. Briefly, 10 μ g of IL-1 type 3 receptor expression plasmid DNA are diluted in 3 ml of Dulbecco's modified Minimum Essential Medium (D-MEM) supplemented with glutamine, pyruvate, 25mM HEPES, 100 microgram/ml DEAE dextran (0.5 Md., Sigma, St. Louis) and 0.1 mM chloroquine (Sigma). Cells are incubated in this transfection mixture for 4 hours at 10 37°C. After one washing step with D-MEM cells are incubated for 48 hours in D-MEM supplemented with 10% fetal calf serum. At this stage cells are ready for further analysis of the expressed IL-1 type 3 receptor.

15

EXAMPLE 4

SIGNALING OF IL-1 VIA THE IL-1 TYPE 3
RECEPTOR IN A FUNCTIONAL ASSAY

IL-1 type 1 receptor cDNA and type 3 receptor cDNA are separately transfected into Jurkat cells (ATCC no. TIB 152) together with a reporter plasmid consisting of the HIV promoter region (HTV-LTR) linked to the bacterial chloramphenicol acetyltransferase (CAT) gene. Stimulation of the transfected cells with human IL-1 alpha leads through a signaling cascade involving the transcription factor NF-kappaB to the production of CAT, which in turn can be measured by commercially available assays (Promega, Madison, WI) (see also Leung et al., *J. Biol. Chem.* 269:1579-1582, 1994).

Results are shown in Figure 3. Briefly, approximately equal stimulation of CAT activity for both receptors can be seen over mock transfected control cells. This indicates that human IL-1 alpha can signal through the IL-1 type 3 receptor.

30

EXAMPLE 5

EXPRESSION, LOCALIZATION, AND ACTIVITY OF THE IL-1 TYPE 3 RECEPTOR

35

A. Expression Pattern of the IL-1 Type 3 Receptor

In order to determine in which rat tissues and parts of the rat brain the IL-1 Type 3 receptor is expressed, RNA protection assays are performed. Briefly, total

RNA is isolated from each tissue or part of the brain and annealed at 65°C to ³²P-labeled RNA generated from a plasmid containing a 600 bp fragment which covers the entire transmembrane region and portions of the extracellular and intracellular domains of the Type 3 receptor cDNA. Samples are then digested with RNase and fractionated 5 on a denaturing polyacrylamide gel. The gel is then dried and the radioactivity quantitated using a Phospholmager (Figure 4).

As can be seen in Figure 4, the highest level of expression is in the lung, followed by the epididymis and testis. When various areas of the brain are examined, the cerebral cortex contains the highest level of the Type 3 receptor, although other 10 areas of the brain were also positive.

B. Localization of the IL-1 Type 3 Receptor by In Situ Hybridization

Utilizing *in situ* hybridization histochemistry, the IL-1 type 3 receptor 15 may be found in the thymus and the spleen. In the thymus the signal is most prominent in the cortical region and not in the medulla. Within the rat brain the IL-1 type 3 receptor expression is detectable in the hippocampus and the fourth ventricle. This is in contrast to the localization of the IL-1 type 1 receptor which is restricted to the dentate gyrus granule cells.

20 Briefly, dissected tissue is frozen in isopentane cooled to -42°C and subsequently stored at -80°C prior to sectioning on a cryostat. Slide-mounted tissue sections are then stored at -80°C. Sections are removed from storage and placed directly into 4% buffered paraformaldehyde at room temperature. After 60 minutes, slides are rinsed in isotonic phosphate buffered saline (10 min.) and treated with 25 proteinase K (1 µg/ml in 100 mM Tris/HCl, pH 8.0) for 10 minutes at 37°C. Subsequently, sections are successively washed in water (1 min.), 0.1 M triethanolamine (pH 8.0, plus 0.25% acetic anhydride) for 10 minutes and 2X SSC (0.3 mM NaCl, 0.03 mM sodium citrate, pH 7.2) for 5 minutes. Sections are then dehydrated through graded alcohols and air dried. Post-fixed sections are hybridized with 1.0 x 10⁶ dpm 30 [³⁵S]UTP-labeled riboprobes in hybridization buffer containing 75% formamide, 10% dextran sulphate, 3X SSC, 50 mM sodium phosphate buffer pH 7.4), 1X Denhardt's solution, 0.1 mg/ml yeast tRNA and 10 mM dithiothreitol in a total volume of 30 µl. The diluted probe is applied to sections on a glass coverslip and hybridized overnight at 55°C in a humid environment. Post-hybridization, sections are washed in 2X SSC for 35 5 minutes and then treated with RNase A (200 µg/ml in 10 mM Tris/HCl, pH 8.0, containing 0.5 M NaCl) for 60 minutes at 37°C. Subsequently, sections are washed in 2X SSC for 5 minutes, 1X SSC for 5 minutes, 0.1X SSC for 60 minutes at 70°C, 0.5X

SSC at room temperature for 5 minutes and then dehydrated in graded alcohols and air dried. For signal detection, sections are placed on Kodak Bio Max X-ray film and exposed for the required length of time or dipped in photographic emulsion (Amersham LM-1) for high resolution analysis. Autoradiograms are analyzed using automated 5 image analysis (DAGE camera/Mac II) while dipped sections were examined using a Zeiss Axioscope.

C. Inhibition of Thymocyte Proliferation by the IL-1 Type 3 Receptor

10 Ability of the IL-1 type 3 receptor to inhibit mouse thymocyte proliferation may also be examined. Briefly, the proliferative response of T lymphocyte lectins such as phytohemagglutin (PHA) is very low, but is markedly enhanced by IL-1. Thus, soluble type human and rat type 3 receptors may be utilized to competitively inhibit proliferation of mouse thymocytes stimulated by IL-1. Soluble human Type 1 15 receptor produced in baculovirus may be used as a positive control.

Briefly, soluble IL-1 type 1 or type 3 receptors are added to wells of a 96 well plate and serially diluted IL-1 is also added. Thymi are removed from young mice and a single cell suspension prepared in tissue culture media. Cells are washed 3 times and resuspended at a concentration of 10^7 cells/ml. Cells are plated at 100 microliters in 20 a 96 well flat bottom microtiter plate. PHA is added to stimulate the cells. Plates are then incubated for 48 hours in a 37°C, 5% CO₂ humidified incubator, and [³H] thymidine is added to the cells for the last 4 to 6 hours. Cells are then harvested and the [³H] thymidine incorporation determined by liquid scintillation counting.

25 As shown in Figure 5, both human IL-1 type 3 and rat IL-1 type 3 receptors effectively inhibit thymocyte proliferation in a manner similar to that observed for soluble human type 1 receptor. This result strongly indicates that the type 3 receptor inhibits thymocyte proliferation by binding to the exogenously added IL-1.

From the foregoing, it will be appreciated that, although specific 30 embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Lovenberg, Timothy W.
Oltersdorf, Tilman
Liaw, Chen W.
Clevenger, William
DeSouza, Errol B.

(ii) TITLE OF INVENTION: INTERLEUKIN-1 TYPE 3 RECEPTORS

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Seed and Berry
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: US
(F) ZIP: 98104

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: McMasters, David D.
(B) REGISTRATION NUMBER: 33,963

(C) REFERENCE/DOCKET NUMBER: 690068.402PC

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (206) 622-4900
- (B) TELEFAX: (206) 682-6031
- (C) TELEX: 3723836

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1965 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 129..1814

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCCCGCCCA CGGGGGCGGG GAAATACCTA GGCGATGGAAG TGGCATGACA GGGCTCGTGT 60

CCCTGTCTATA TTTTCCACTC TCCACGAGGT CCTGCGGGCT TCAATCCTGC AGGCAGCCCG 120

GTGGGGGG ATG TGG TCC TTG CTG CTC TGC GGG TTG TCC ATC GCC CTT CCA 170
Met Trp Ser Leu Leu Leu Cys Gly Leu Ser Ile Ala Leu Pro

1 5 10

CTG TCT GTC ACA GCA GAT GGA TGC AAG GAC ATT TTT ATG AAA AAT GAG 218
Leu Ser Val Thr Ala Asp Gly Cys Lys Asp Ile Phe Met Lys Asn Glu
15 20 25 30

ATA CTT TCA GCA AGC CAG CCT TTT GCT TTT AAT TGT ACA TTC CCT CCC 266

Ile Leu Ser Ala Ser Gln Pro Phe Ala Phe Asn Cys Thr Phe Pro Pro
 35 40 45

ATA ACA TCT GGG GAA GTC AGT GTA ACA TGG TAT AAA AAT TCT AGC AAA 314
 Ile Thr Ser Gly Glu Val Ser Val Thr Trp Tyr Lys Asn Ser Ser Lys
 50 55 60

ATC CCA GTG TCC AAA ATC ATA CAG TCT AGA ATT CAC CAG GAC GAG ACT 362
 Ile Pro Val Ser Lys Ile Ile Gln Ser Arg Ile His Gln Asp Glu Thr
 65 70 75

TGG ATT TTG TTT CTC CCC ATG GAA TGG GGG GAC TCA GGA GTC TAC CAA 410
 Trp Ile Leu Phe Leu Pro Met Glu Trp Gly Asp Ser Gly Val Tyr Gln
 80 85 90

TGT GTT ATA AAG GGT AGA GAC AGC TGT CAT AGA ATA CAT GTA AAC CTA 458
 Cys Val Ile Lys Gly Arg Asp Ser Cys His Arg Ile His Val Asn Leu
 95 100 105 110

ACT GTT TTT GAA AAA CAT TGG TGT GAC ACT TCC ATA GGT GGT TTA CCA 506
 Thr Val Phe Glu Lys His Trp Cys Asp Thr Ser Ile Gly Gly Leu Pro
 115 120 125

AAT TTA TCA GAT GAG TAC AAG CAA ATA TTA CAT CTT GGA AAA GAT GAT 554
 Asn Leu Ser Asp Glu Tyr Lys Gln Ile Leu His Leu Gly Lys Asp Asp
 130 135 140

AGT CTC ACA TGT CAT CTG CAC TTC CCG AAG AGT TGT GTT TTG GGT CCA 602
 Ser Leu Thr Cys His Leu His Phe Pro Lys Ser Cys Val Leu Gly Pro
 145 150 155

ATA AAG TGG TAT AAG GAC TGT AAC GAG ATT AAA GGG GAG CGG TTC ACT 650
 Ile Lys Trp Tyr Lys Asp Cys Asn Glu Ile Lys Gly Glu Arg Phe Thr
 160 165 170

GTT TTG GAA ACC AGG CTT TTG GTG AGC AAT GTC TCG GCA GAG GAC AGA 698
 Val Leu Glu Thr Arg Leu Leu Val Ser Asn Val Ser Ala Glu Asp Arg

175

180

185

190

GGG AAC TAC GCG TGT CAA GCC ATA CTG ACA CAC TCA GGG AAG CAG TAC
 Gly Asn Tyr Ala Cys Gln Ala Ile Leu Thr His Ser Gly Lys Gln Tyr
 195 200 205

746

GAG GTT TTA AAT GGC ATC ACT GTG AGC ATT ACA GAA AGA GCT GGA TAT
 Glu Val Leu Asn Gly Ile Thr Val Ser Ile Thr Glu Arg Ala Gly Tyr
 210 215 220

794

GGA GGA AGT GTC CCT AAA ATC ATT TAT CCA AAA AAT CAT TCA ATT GAA
 Gly Gly Ser Val Pro Lys Ile Ile Tyr Pro Lys Asn His Ser Ile Glu
 225 230 235

842

GTA CAG CTT GGT ACC ACT CTG ATT GTG GAC TGC AAT GTA ACA GAC ACC
 Val Gln Leu Gly Thr Thr Leu Ile Val Asp Cys Asn Val Thr Asp Thr
 240 245 250

890

AAG GAT AAT ACA AAT CTA CGA TGC TGG AGA GTC AAT AAC ACT TTG GTG
 Lys Asp Asn Thr Asn Leu Arg Cys Trp Arg Val Asn Asn Thr Leu Val
 255 260 265 270

938

GAT GAT TAC TAT GAT GAA TCC AAA CGA ATC AGA GAA GGG GTG GAA ACC
 Asp Asp Tyr Tyr Asp Glu Ser Lys Arg Ile Arg Glu Gly Val Glu Thr
 275 280 285

986

CAT GTC TCT TTT CGG GAA CAT AAT TTG TAC ACA GTA AAC ATC ACC TTC
 His Val Ser Phe Arg Glu His Asn Leu Tyr Thr Val Asn Ile Thr Phe
 290 295 300

1034

TTG GAA GTG AAA ATG GAA GAT TAT GGC CTT CCT TTC ATG TGC CAC GCT
 Leu Glu Val Lys Met Glu Asp Tyr Gly Leu Pro Phe Met Cys His Ala
 305 310 315

1082

GGA GTG TCC ACA GCA TAC ATT ATA TTA CAG CTC CCA GCT CCG GAT TTT
 Gly Val Ser Thr Ala Tyr Ile Ile Leu Gln Leu Pro Ala Pro Asp Phe
 320 325 330

1130

CGA GCT TAC TTG ATA GGA GGG CTT ATC GCC TTG GTG GCT GTG GCT GTG			1178
Arg Ala Tyr Leu Ile Gly Gly Leu Ile Ala Leu Val Ala Val Ala Val			
335	340	345	350
TCT GTT GTG TAC ATA TAC AAC ATT TTT AAG ATC GAC ATT GTT CTT TGG			1226
Ser Val Val Tyr Ile Tyr Asn Ile Phe Lys Ile Asp Ile Val Leu Trp			
355	360	365	
TAT CGA AGT GCC TTC CAT TCT ACA GAG ACC ATA GTA GAT GGG AAG CTG			1274
Tyr Arg Ser Ala Phe His Ser Thr Glu Thr Ile Val Asp Gly Lys Leu			
370	375	380	
TAT GAC GCC TAT GTC TTA TAC CCC AAG CCC CAC AAG GAA AGC CAG AGG			1322
Tyr Asp Ala Tyr Val Leu Tyr Pro Lys Pro His Lys Glu Ser Gln Arg			
385	390	395	
CAT GCC GTG GAT GCC CTG GTG TTG AAT ATC CTG CCC GAG GTG TTG GAG			1370
His Ala Val Asp Ala Leu Val Leu Asn Ile Leu Pro Glu Val Leu Glu			
400	405	410	
AGA CAA TGT GGA TAT AAG TTG TTT ATA TTC GGC AGA GAT GAA TTC CCT			1418
Arg Gln Cys Gly Tyr Lys Leu Phe Ile Phe Gly Arg Asp Glu Phe Pro			
415	420	425	430
GGA CAA GCC GTG GCC AAT GTC ATC GAT GAA AAC GTT AAG CTG TGC AGG			1466
Gly Gln Ala Val Ala Asn Val Ile Asp Glu Asn Val Lys Leu Cys Arg			
435	440	445	
AGG CTG ATT GTC ATT GTG GTC CCC GAA TCG CTG GGC TTT GGC CTG TTG			1514
Arg Leu Ile Val Ile Val Val Pro Glu Ser Leu Gly Phe Gly Leu Leu			
450	455	460	
AAG AAC CTG TCA GAA GAA CAA ATC GCG GTC TAC AGT GCC CTG ATC CAG			1562
Lys Asn Leu Ser Glu Glu Gln Ile Ala Val Tyr Ser Ala Leu Ile Gln			
465	470	475	

GAC GGG ATG AAG GTT ATT CTC ATT GAG CTG GAG AAA ATC GAG GAC TAC 1610
 Asp Gly Met Lys Val Ile Leu Ile Glu Leu Lys Ile Glu Asp Tyr
 480 485 490

ACA GTC ATG CCA GAG TCA ATT CAG TAC ATC AAA CAG AAG CAT GGT GCC 1658
 Thr Val Met Pro Glu Ser Ile Gln Tyr Ile Lys Gln Lys His Gly Ala
 495 500 505 510

ATC CGG TGG CAT GGG GAC TTC ACG GAG CAG TCA CAG TGT ATG AAG ACC 1706
 Ile Arg Trp His Gly Asp Phe Thr Glu Gln Ser Gln Cys Met Lys Thr
 515 520 525

AAG TTT TGG AAG ACA GTG AGA TAC CAC ATG CCG CCC AGA AGG TGT CGG 1754
 Lys Phe Trp Lys Thr Val Arg Tyr His Met Pro Pro Arg Arg Cys Arg
 530 535 540

CCG TTT CTC CGG TCC ACG TGC CGC AGC ACA CAC CTC TGT ACC GCA CCG 1802
 Pro Phe Leu Arg Ser Thr Cys Arg Ser Thr His Leu Cys Thr Ala Pro
 545 550 555

CAG GCC CAG AAC TAGGCTCAAG AAGAAAGAAG TGTACTCTCA CGACTGGCTA 1854
 Gln Ala Gln Asn
 560

AGACTTGCTG GACTGACACC TATGGCTGGA AGATGACTTG TTTTGCTCCA TGTCTCCTCA 1914

TTCCTACACC TATTTCTGC TGCAGGGATGA GGCTAGGGTT AGCATTCTAG A 1965

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 562 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Trp Ser Leu Leu Leu Cys Gly Leu Ser Ile Ala Leu Pro Leu Ser
1 5 10 15

Val Thr Ala Asp Gly Cys Lys Asp Ile Phe Met Lys Asn Glu Ile Leu
20 25 30

Ser Ala Ser Gln Pro Phe Ala Phe Asn Cys Thr Phe Pro Pro Ile Thr
35 40 45

Ser Gly Glu Val Ser Val Thr Trp Tyr Lys Asn Ser Ser Lys Ile Pro
50 55 60

Val Ser Lys Ile Ile Gln Ser Arg Ile His Gln Asp Glu Thr Trp Ile
65 70 75 80

Leu Phe Leu Pro Met Glu Trp Gly Asp Ser Gly Val Tyr Gln Cys Val
85 90 95

Ile Lys Gly Arg Asp Ser Cys His Arg Ile His Val Asn Leu Thr Val
100 105 110

Phe Glu Lys His Trp Cys Asp Thr Ser Ile Gly Gly Leu Pro Asn Leu
115 120 125

Ser Asp Glu Tyr Lys Gln Ile Leu His Leu Gly Lys Asp Asp Ser Leu
130 135 140

Thr Cys His Leu His Phe Pro Lys Ser Cys Val Leu Gly Pro Ile Lys
145 150 155 160

Trp Tyr Lys Asp Cys Asn Glu Ile Lys Gly Glu Arg Phe Thr Val Leu
165 170 175

Glu Thr Arg Leu Leu Val Ser Asn Val Ser Ala Glu Asp Arg Gly Asn

180 185 190

Tyr Ala Cys Gln Ala Ile Leu Thr His Ser Gly Lys Gln Tyr Glu Val
195 200 205

Leu Asn Gly Ile Thr Val Ser Ile Thr Glu Arg Ala Gly Tyr Gly Gly
210 215 220

Ser Val Pro Lys Ile Ile Tyr Pro Lys Asn His Ser Ile Glu Val Gln
225 230 235 240

Leu Gly Thr Thr Leu Ile Val Asp Cys Asn Val Thr Asp Thr Lys Asp
245 250 255

Asn Thr Asn Leu Arg Cys Trp Arg Val Asn Asn Thr Leu Val Asp Asp
260 265 270

Tyr Tyr Asp Glu Ser Lys Arg Ile Arg Glu Gly Val Glu Thr His Val
275 280 285

Ser Phe Arg Glu His Asn Leu Tyr Thr Val Asn Ile Thr Phe Leu Glu
290 295 300

Val Lys Met Glu Asp Tyr Gly Leu Pro Phe Met Cys His Ala Gly Val
305 310 315 320

Ser Thr Ala Tyr Ile Ile Leu Gln Leu Pro Ala Pro Asp Phe Arg Ala
325 330 335

Tyr Leu Ile Gly Gly Leu Ile Ala Leu Val Ala Val Ala Val Ser Val
340 345 350

Val Tyr Ile Tyr Asn Ile Phe Lys Ile Asp Ile Val Leu Trp Tyr Arg
355 360 365

Ser Ala Phe His Ser Thr Glu Thr Ile Val Asp Gly Lys Leu Tyr Asp
370 375 380

Ala Tyr Val Leu Tyr Pro Lys Pro His Lys Glu Ser Gln Arg His Ala
385 390 395 400

Val Asp Ala Leu Val Leu Asn Ile Leu Pro Glu Val Leu Glu Arg Gln
405 410 415

Cys Gly Tyr Lys Leu Phe Ile Phe Gly Arg Asp Glu Phe Pro Gly Gln
420 425 430

Ala Val Ala Asn Val Ile Asp Glu Asn Val Lys Leu Cys Arg Arg Leu
435 440 445

Ile Val Ile Val Val Pro Glu Ser Leu Gly Phe Gly Leu Leu Lys Asn
450 455 460

Leu Ser Glu Glu Gln Ile Ala Val Tyr Ser Ala Leu Ile Gln Asp Gly
465 470 475 480

Met Lys Val Ile Leu Ile Glu Leu Glu Lys Ile Glu Asp Tyr Thr Val
485 490 495

Met Pro Glu Ser Ile Gln Tyr Ile Lys Gln Lys His Gly Ala Ile Arg
500 505 510

Trp His Gly Asp Phe Thr Glu Gln Ser Gln Cys Met Lys Thr Lys Phe
515 520 525

Trp Lys Thr Val Arg Tyr His Met Pro Pro Arg Arg Cys Arg Pro Phe
530 535 540

Leu Arg Ser Thr Cys Arg Ser Thr His Leu Cys Thr Ala Pro Gln Ala
545 550 555 560

Gln Asn

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2044 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 89..1771

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGGCTGGCC TAGGATCAGG CAAGAAAAGG CTGAAACGCCT TTCTAAGGAC GGACTCTTC	60
TGTACAGCTC CACTGGGGA AGCCCGAA ATG GGG ATG CCA CCC TTG CTC TTC	
Met Gly Met Pro Pro Leu Leu Phe	
1	5
TGT TGG GTG TCT GTG CTT CCA CTT TTT GTG GCA GCA GGT AAC TGT	
Cys Trp Val Ser Phe Val Leu Pro Leu Phe Val Ala Ala Gly Asn Cys	
10	15
20	
ACT GAT GTC TAT ATG CAC CAT GAG ATG ATT TCA GAG GGC CAG CCT TTC	
Thr Asp Val Tyr Met His His Glu Met Ile Ser Glu Gly Gln Pro Phe	
25	30
35	
40	
CCC TTC AAC TGC ACA TAC CCT CCA GTA ACA AAC GGG GCA GTG AAT CTG	
Pro Phe Asn Cys Thr Tyr Pro Pro Val Thr Asn Gly Ala Val Asn Leu	
45	50
55	
ACA TGG CAT AGA ACA CCC AGT AAG AGC CCA ATC TCC ATC AAC AGA CAC	
Thr Trp His Arg Thr Pro Ser Lys Ser Pro Ile Ser Ile Asn Arg His	
60	65
70	

GTT AGA ATT CAC CAG GAC CAG TCC TGG ATT TTG TTT CTT CCG TTG GCA Val Arg Ile His Gln Asp Gln Ser Trp Ile Leu Phe Leu Pro Leu Ala	75	80	85	352
TTG GAG GAC TCA GGC ATC TAT CAA TGT GTT ATA AAG GAT GCC CAC AGC Leu Glu Asp Ser Gly Ile Tyr Gln Cys Val Ile Lys Asp Ala His Ser	90	95	100	400
TGT TAC CGA ATA GCT ATA AAC CTA ACC GTT TTT AGA AAA CAC TGG TGC Cys Tyr Arg Ile Ala Ile Asn Leu Thr Val Phe Arg Lys His Trp Cys	105	110	115	448
GAC TCT TCC AAC GAA GAG AGT TCC ATA AAT TCC TCA GAT GAG TAC CAG Asp Ser Ser Asn Glu Glu Ser Ser Ile Asn Ser Ser Asp Glu Tyr Gln	125	130	135	496
CAA TGG TTA CCC ATA GGA AAA TCG GGC AGT CTG ACG TGC CAT CTC TAC Gln Trp Leu Pro Ile Gly Lys Ser Gly Ser Leu Thr Cys His Leu Tyr	140	145	150	544
TTC CCA GAG AGC TGT GTT TTG GAT TCA ATA AAG TGG TAT AAG GGT TGT Phe Pro Glu Ser Cys Val Leu Asp Ser Ile Lys Trp Tyr Lys Gly Cys	155	160	165	592
GAA GAG ATT AAA GTG AGC AAG AAG TTT TGC CCT ACA GGA ACA AAG CTT Glu Glu Ile Lys Val Ser Lys Lys Phe Cys Pro Thr Gly Thr Lys Leu	170	175	180	640
CTT GTT AAC AAC ATC GAC GTG GAG GAT AGT GGG AGC TAT GCA TGC TCA Leu Val Asn Asn Ile Asp Val Glu Asp Ser Gly Ser Tyr Ala Cys Ser	185	190	195	688
GCC AGA CTG ACA CAC TTG GGG AGA ATC TTC ACG GTT AGA AAC TAC ATT Ala Arg Leu Thr His Leu Gly Arg Ile Phe Thr Val Arg Asn Tyr Ile	205	210	215	736

GCT GTG AAT ACC AAG GAA GTT GGG TCT GGA GGA AGG ATC CCT AAC ATC			784
Ala Val Asn Thr Lys Glu Val Gly Ser Gly Gly Arg Ile Pro Asn Ile			
220	225	230	
ACG TAT CCA AAA AAC AAC TCC ATT GAA GTT CAA CTT GGC TCC ACC CTC			832
Thr Tyr Pro Lys Asn Asn Ser Ile Glu Val Gln Leu Gly Ser Thr Leu			
235	240	245	
ATT GTG GAC TGC AAT ATA ACA GAC ACG AAG GAG AAT ACG AAC CTC AGA			880
Ile Val Asp Cys Asn Ile Thr Asp Thr Lys Glu Asn Thr Asn Leu Arg			
250	255	260	
TGC TGG CGA GTT AAC AAC ACC CTG GTG GAC GAT TAC TAC AAC GAC TTC			928
Cys Trp Arg Val Asn Asn Thr Leu Val Asp Asp Tyr Tyr Asn Asp Phe			
265	270	275	280
AAA CGC ATC CAG GAA GGA ATC GAA ACC AAT CTG TCT CTG AGG AAT CAC			976
Lys Arg Ile Gln Glu Gly Ile Glu Thr Asn Leu Ser Leu Arg Asn His			
285	290	295	
ATT CTG TAC ACA GTG AAC ATA ACA TTC TTA GAA GTG AAA ATG GAG GAC			1024
Ile Leu Tyr Thr Val Asn Ile Thr Phe Leu Glu Val Lys Met Glu Asp			
300	305	310	
TAC GGC CAT CCT TTC ACA TGC CAC GCT GCG GTG TCC GCA GCC TAC ATC			1072
Tyr Gly His Pro Phe Thr Cys His Ala Ala Val Ser Ala Ala Tyr Ile			
315	320	325	
ATT CTG AAA CGC CCA GCT CCA GAC TTC CGG GCT TAC CTC ATA GGA GGT			1120
Ile Leu Lys Arg Pro Ala Pro Asp Phe Arg Ala Tyr Leu Ile Gly Gly			
330	335	340	
CTC ATG GCT TTC CTA CTT CTG GCC GTG ATT CTG TAC ATC TAC AAC			1168
Leu Met Ala Phe Leu Leu Ala Val Ser Ile Leu Tyr Ile Tyr Asn			
345	350	355	360
ACC TTT AAG GTC GAC ATC GTG CTT TGG TAT AGG AGT ACC TTC CAC ACT			1216

Thr Phe Lys Val Asp Ile Val Leu Trp Tyr Arg Ser Thr Phe His Thr			
365	370	375	
GCC CAG GCT CCA GAT GAC GAG AAG CTG TAT GAT GCC TAT GTC TTA TAC			
Ala Gln Ala Pro Asp Asp Glu Lys Leu Tyr Asp Ala Tyr Val Leu Tyr			1264
380	385	390	
CCC AAG TAC CCA AGA GAA AGC CAG GGC CAT GAT GTG GAC ACA CTG GTG			
Pro Lys Tyr Pro Arg Glu Ser Gln Gly His Asp Val Asp Thr Leu Val			1312
395	400	405	
TTG AAG ATC TTG CCC GAG GTG CTG GAG AAA CAG TGT GGA TAT AAG TTA			
Leu Lys Ile Leu Pro Glu Val Leu Glu Lys Gln Cys Gly Tyr Lys Leu			1360
410	415	420	
TTC ATA TTT GGC AGG GAT GAA TTC CCT GGA CAA GCT GTG GCC AGC GTC			
Phe Ile Phe Gly Arg Asp Glu Phe Pro Gly Gln Ala Val Ala Ser Val			1408
425	430	435	440
ATT GAT GAA AAC ATT AAG CTG TGT AGG AGG CTG ATG GTC CTC GTG GCA			
Ile Asp Glu Asn Ile Lys Leu Cys Arg Arg Leu Met Val Leu Val Ala			1456
445	450	455	
CCA GAG ACA TCC AGC TTC AGC TTT CTG AAG AAC TTG ACT GAA GAA CAA			
Pro Glu Thr Ser Ser Phe Ser Phe Leu Lys Asn Leu Thr Glu Glu Gln			1504
460	465	470	
ATC GCT GTC TAC AAT GCC CTC GTC CAG GAC GGC ATG AAG GTC ATT CTG			
Ile Ala Val Tyr Asn Ala Leu Val Gln Asp Gly Met Lys Val Ile Leu			1552
475	480	485	
ATT GAA CTG GAG AGA GTC AAG GAC TAC AGC ACC ATG CCC GAG TCC ATT			
Ile Glu Leu Glu Arg Val Lys Asp Tyr Ser Thr Met Pro Glu Ser Ile			1600
490	495	500	
CAG TAC ATC CGA CAG AAG CAC GGG GCC ATC CAG TGG GAT GGG GAC TTC			
Gln Tyr Ile Arg Gln Lys His Gln Ala Ile Gln Trp Asp Gly Asp Phe			1648

505	510	515	520		
ACA GAG CAG GCA CAG TGC GCC AAG ACG AAA TTC TGG AAG AAA GTG AGA					1696
Thr Glu Gln Ala Gln Cys Ala Lys Thr Lys Phe Trp Lys Lys Val Arg					
525	530	535			
TAT CAT ATG CCA CCC AGG AGG TAC CCG GCA TCT CCC CCC GTC CAG CTG					1744
Tyr His Met Pro Pro Arg Arg Tyr Pro Ala Ser Pro Pro Val Gln Leu					
540	545	550			
CTA GGA CAC ACA CCC CGC ATA CCA GGC TAGTGCAGTG CCACCGCCAC					1791
Leu Gly His Thr Pro Arg Ile Pro Gly					
555	560				
GGGGCTCATA ACTCCTTAAG AGCGGTTAGT GTGTGGTGGC TCGCACTACA ACCTCTCTGG					1851
ATCATCTACC CCCGTAGCTT GCTCTTTGT GCTTGTGAGC GACCTCGTCC TTAGCCACGT					1911
CATATTTGTA TTTTTGTTT GTTTGTTTG TTTGTTGTAT GCTTTAGTC ATAGCTGATT					1971
CGTACTACTC CTGTTTGCTT CATGGTTCCCT GAATCCCAGA GACTCCCTGA GCATGGGTGG					2031
CTATCATGTT GGG					2044

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 561 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Met Pro Pro Leu Leu Phe Cys Trp Val Ser Phe Val Leu Pro

50

1

5

10

15

Leu Phe Val Ala Ala Gly Asn Cys Thr Asp Val Tyr Met His His Glu

20

25

30

Met Ile Ser Glu Gly Gln Pro Phe Pro Phe Asn Cys Thr Tyr Pro Pro

35

40

45

Val Thr Asn Gly Ala Val Asn Leu Thr Trp His Arg Thr Pro Ser Lys

50

55

60

Ser Pro Ile Ser Ile Asn Arg His Val Arg Ile His Gln Asp Gln Ser

65

70

75

80

Trp Ile Leu Phe Leu Pro Leu Ala Leu Glu Asp Ser Gly Ile Tyr Gln

85

90

95

Cys Val Ile Lys Asp Ala His Ser Cys Tyr Arg Ile Ala Ile Asn Leu

100

105

110

Thr Val Phe Arg Lys His Trp Cys Asp Ser Ser Asn Glu Glu Ser Ser

115

120

125

Ile Asn Ser Ser Asp Glu Tyr Gln Gln Trp Leu Pro Ile Gly Lys Ser

130

135

140

Gly Ser Leu Thr Cys His Leu Tyr Phe Pro Glu Ser Cys Val Leu Asp

145

150

155

160

Ser Ile Lys Trp Tyr Lys Gly Cys Glu Glu Ile Lys Val Ser Lys Lys

165

170

175

Phe Cys Pro Thr Gly Thr Lys Leu Leu Val Asn Asn Ile Asp Val Glu

180

185

190

Asp Ser Gly Ser Tyr Ala Cys Ser Ala Arg Leu Thr His Leu Gly Arg

195

200

205

Ile Phe Thr Val Arg Asn Tyr Ile Ala Val Asn Thr Lys Glu Val Gly
210 215 220

Ser Gly Gly Arg Ile Pro Asn Ile Thr Tyr Pro Lys Asn Asn Ser Ile
225 230 235 240

Glu Val Gln Leu Gly Ser Thr Leu Ile Val Asp Cys Asn Ile Thr Asp
245 250 255

Thr Lys Glu Asn Thr Asn Leu Arg Cys Trp Arg Val Asn Asn Thr Leu
260 265 270

Val Asp Asp Tyr Tyr Asn Asp Phe Lys Arg Ile Gln Glu Gly Ile Glu
275 280 285

Thr Asn Leu Ser Leu Arg Asn His Ile Leu Tyr Thr Val Asn Ile Thr
290 295 300

Phe Leu Glu Val Lys Met Glu Asp Tyr Gly His Pro Phe Thr Cys His
305 310 315 320

Ala Ala Val Ser Ala Ala Tyr Ile Ile Leu Lys Arg Pro Ala Pro Asp
325 330 335

Phe Arg Ala Tyr Leu Ile Gly Gly Leu Met Ala Phe Leu Leu Ala
340 345 350

Val Ser Ile Leu Tyr Ile Tyr Asn Thr Phe Lys Val Asp Ile Val Leu
355 360 365

Trp Tyr Arg Ser Thr Phe His Thr Ala Gln Ala Pro Asp Asp Glu Lys
370 375 380

Leu Tyr Asp Ala Tyr Val Leu Tyr Pro Lys Tyr Pro Arg Glu Ser Gln
385 390 395 400

52

Gly His Asp Val Asp Thr Leu Val Leu Lys Ile Leu Pro Glu Val Leu
405 410 415

Glu Lys Gln Cys Gly Tyr Lys Leu Phe Ile Phe Gly Arg Asp Glu Phe
420 425 430

Pro Gly Gln Ala Val Ala Ser Val Ile Asp Glu Asn Ile Lys Leu Cys
435 440 445

Arg Arg Leu Met Val Leu Val Ala Pro Glu Thr Ser Ser Phe Ser Phe
450 455 460

Leu Lys Asn Leu Thr Glu Glu Gln Ile Ala Val Tyr Asn Ala Leu Val
465 470 475 480

Gln Asp Gly Met Lys Val Ile Leu Ile Glu Leu Glu Arg Val Lys Asp
485 490 495

Tyr Ser Thr Met Pro Glu Ser Ile Gln Tyr Ile Arg Gln Lys His Gly
500 505 510

Ala Ile Gln Trp Asp Gly Asp Phe Thr Glu Gln Ala Gln Cys Ala Lys
515 520 525

Thr Lys Phe Trp Lys Val Arg Tyr His Met Pro Pro Arg Arg Tyr
530 535 540

Pro Ala Ser Pro Pro Val Gln Leu Leu Gly His Thr Pro Arg Ile Pro
545 550 555 560

Gly

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 50 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTTCAACTGC ACATAACCCTC CAGTAACAAA CGGGGGCAGTG AATCTGACAT

50

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 51 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTCCCATAA CATCTGGGGA AGTCAGTGTA ACATGGTATA AAAATTCTAG C

51

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTACTCGAG ATGTGGTCCT TGCTGCTC

28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGCGCGGCC GCCTATCGAA AATCCGGAGC TGG

33

Claims

1. An isolated nucleic acid molecule encoding an Interleukin-1 Type 3 receptor.
2. The isolated nucleic acid molecule according to claim 1, comprising the sequence of nucleotides in Sequence I.D. No. 1, from nucleotide number 129 to nucleotide number 1814.
3. The isolated nucleic acid molecule according to claim 1 wherein said molecule encodes a protein having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 562.
4. The isolated nucleic acid molecule according to claim 1, comprising the sequence of nucleotides in Sequence I.D. No. 3, from nucleotide number 89 to nucleotide number 1771.
5. The isolated nucleic acid molecule according to claim 1 wherein said molecule encodes a protein having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 561.
6. The isolated nucleic acid molecule according to claim 1 wherein said molecule encodes a human Interleukin-1 Type 3 receptor.
7. The isolated nucleic acid molecule according to claim 1 wherein said molecule encodes a rat Interleukin-1 Type 3 receptor.
8. An isolated nucleic acid molecule encoding soluble Interleukin-1 Type 3 receptor.
9. The isolated nucleic acid molecule according to claim 8, comprising the sequence of nucleotides in Sequence I.D. No. 1, from nucleotide number 129 to nucleotide number 1136.

10. The isolated nucleic acid molecule according to claim 8 wherein said molecule encodes a protein having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 336.

11. The isolated nucleic acid molecule according to claim 8, comprising the sequence of nucleotides in Sequence I.D. No. 3, from nucleotide number 89 to nucleotide number 1102.

12. The isolated nucleic acid molecule according to claim 8 wherein said molecule encodes a protein having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 338.

13. The isolated nucleic acid molecule according to claim 8 wherein said molecule encodes a soluble human Interleukin-1 Type 3 receptor.

14. The isolated nucleic acid molecule according to claim 8 wherein said molecule encodes a soluble rat Interleukin-1 Type 3 receptor.

15. A recombinant expression vector, comprising a promoter operably linked to a nucleic acid molecule according to any one of claims 1-14.

16. A recombinant viral vector capable of directing the expression of a nucleic acid molecule according to any one of claims 1-14 wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and herpes simplex virus vectors.

17. A host cell containing a recombinant vector according to any one of claims 15 or 16.

18. An isolated Interleukin-1 Type 3 receptor.

19. The isolated Interleukin-1 Type 3 receptor according to claim 18 having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 562.

20. The isolated Interleukin-1 Type 3 receptor according to claim 18 having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 561.

21. The isolated Interleukin-1 Type 3 receptor according to claim 18 wherein said receptor is a human Interleukin-1 Type 3 receptor.

22. The isolated Interleukin-1 Type 3 receptor according to claim 18 wherein said receptor is a rat Interleukin-1 Type 3 receptor.

23. An isolated soluble Interleukin-1 Type 3 receptor.

24. The isolated soluble Interleukin-1 Type 3 receptor according to claim 23 having the amino acid sequence of Sequence I.D. No. 2, from amino acid number 1 to amino acid number 336.

25. The isolated soluble Interleukin-1 Type 3 receptor according to claim 23 having the amino acid sequence of Sequence I.D. No. 4, from amino acid number 1 to amino acid number 338.

26. The isolated soluble Interleukin-1 Type 3 receptor according to claim 23 wherein said receptor is a human Interleukin-1 Type 3 receptor.

27. The isolated soluble Interleukin-1 Type 3 receptor according to claim 23 wherein said receptor is a rat Interleukin-1 Type 3 receptor.

28. An isolated antibody capable of specifically binding to an Interleukin-1 Type 3 receptor.

29. The antibody according to claim 28 wherein said antibody is selected from the group consisting of polyclonal antibodies, monoclonal antibodies, and antibody fragments.

30. The antibody according to claim 28 wherein said antibody is capable of blocking the binding of IL-1 to an Interleukin-1 Type 3 receptor.

31. The antibody according to claim 28 wherein said antibody is selected from the group consisting of murine and human antibodies.

32. A hybridoma which produces an antibody according to any one of claims 28-31.

33. A nucleic acid probe of at least 18 nucleotides in length which is capable of specifically hybridizing to a nucleic acid sequence encoding an Interleukin-1 Type 3 receptor.

1/4

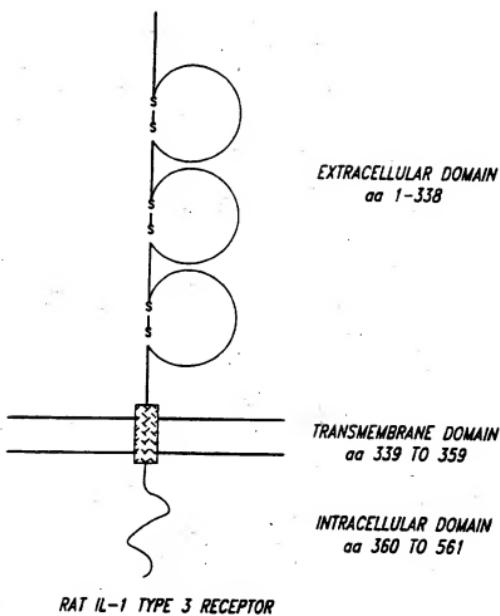


Fig. 1

2/4

HOMOLOGY OF THE HUMAN IL-1 TYPE 3 RECEPTOR WITH RELATED RECEPTORS

		OVERALL	EXTRACELLULAR	INTRACELLULAR	MEMBRANE
RAT	IL-1 R3	66	63	70	66
HUMAN	IL-1 R1	42	34	55	60
RAT	IL-1 R1	40	34	52	60
MOUSE	IL-1 R1	40			
HUMAN	IL-1 R2	23			
MOUSE	IL-1 R2	23			
MOUSE	ST-2L	29			

Fig. 2

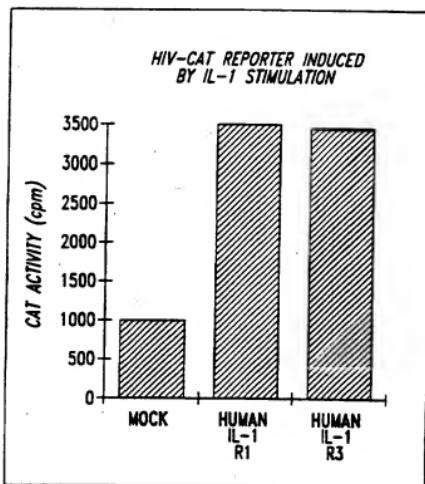


Fig. 3

SUBSTITUTE SHEET (RULE 26)

3/4

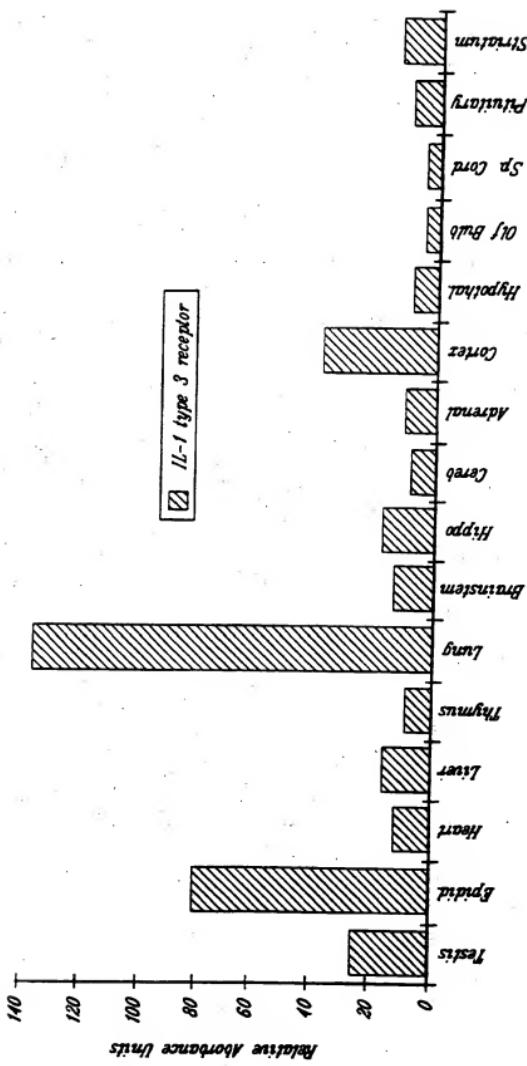


Fig. 4

4/4

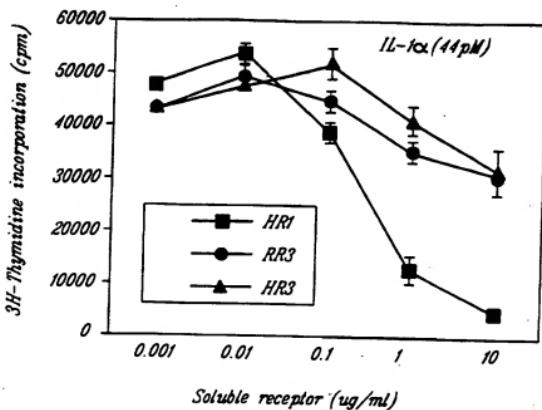


Fig. 5A

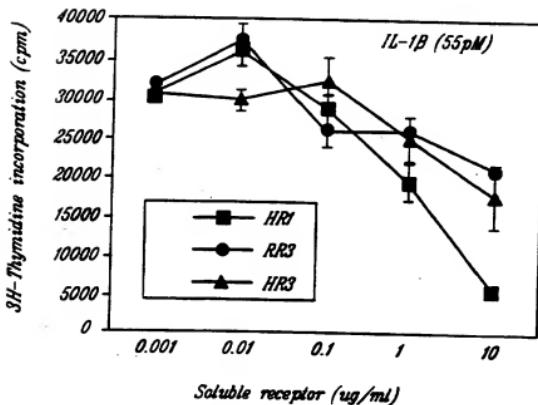


Fig. 5B

SUBSTITUTE SHEET (RULE 26)